JOURNAL OF DAIRY SCIENCE

MAY, 1956

In this issue:

Program of the fifty-first annual meeting

Nomenclature of the proteins of milk—a committee report

Flavor in the quality yardstick

Research articles on manufacturing and production topics

Association and industry news

50th ANNIVERSARY MEETING

June 19-21, '56

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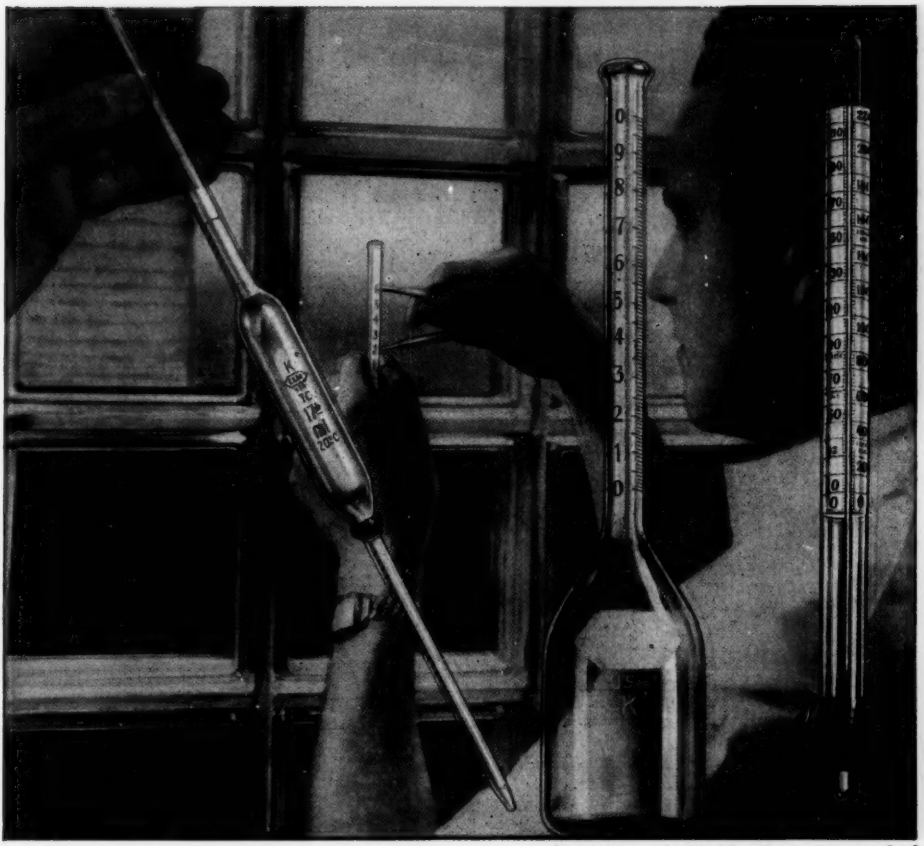

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Changes of Address

Changes of mailing addresses, post office notices of undeliverable copies, inquiries about copies lost in the mail, requests for back copies, and all other matters pertaining to the mailing list should be sent to the secretary-treasurer, H. F. Judkins, American Dairy Science Association, 32 Ridgeway Circle, White Plains, N. Y.

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Subscriptions for the British Isles and the British Empire should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Street, Covent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert Street, St. Leonards, N.S.W., Australia.

Membership dues in the Association are $10.00 a year and should be sent to the secretary-treasurer at the address above. Renewals should be made not later than December 1 each year.

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PEOPLE and EVENTS
in the Dairy Science World

Pioneers of the Dairy Industry

HOWARD BOWMAN ELLENBERGER has crowded much into an interesting career. He has been a student, practical farmer, traveler, administrator, teacher, and scientist. He was born in Dallas, Iowa, and educated in the public schools of that state. He earned the Bachelor of Science degree at Iowa State College in 1905, specializing in the animal sciences. He married Priscilla Flatt in 1910. She passed away in 1952.

H. B. Ellenberger

Dr. Ellenberger was foreman and superintendent of farms in Illinois, Missouri, and Iowa. In 1914 he enrolled in the graduate school of Cornell University, where he was later appointed instructor. He earned the degree of Master of Science in 1915 and that of Doctor of Philosophy in 1917. He has the distinction of being one of the first scientists in this country to earn the highest degree in the field of dairying. While doing graduate work at Cornell University, he worked during his summer vacations as dairy manufacturing specialist for the Vermont Department of Agriculture. His work attracted the attention of Dean J. L. Hills at the University of Vermont, and he was offered and accepted the position of associate professor of animal and dairy husbandry in the College of Agriculture in 1917 and was made head of the department in 1918. His immediate job was to expand the staff in order to better serve the dairy interests in the state. In 1927 his efforts were rewarded with a substantial appropriation from the Vermont legislature for an expanded research, teaching, and extension program with modern facilities. He was a driving force through the years to obtain a new dairy building, which was first occupied in 1948.

With his undergraduate training in the animal sciences, with practical farm experience, and with graduate training in bacteriology and manufacturing, he was admirably fitted to study the problems and needs of the dairy farmer and of industry in Vermont and in New England. He initiated sound programs in extension and research, in plant and dairy herd management, in quality control of dairy products, in regulatory work, and in animal nutrition. Some of his researches in animal nutrition covered several generations of dairy cattle. He is author and co-author of many bulletins and scientific papers in all of these areas and has published seven technical bulletins since retiring in 1948.

Dr. Ellenberger has always been active in trade and professional association work. He served as chairman of the Eastern Division of the A.D.S.A. in 1926, chairman of the Production Section in 1930, president of the National Association in 1931, and director in 1940-42, and has served on a number of committees. In 1949 this association selected him for the distinguished award, "Dairyman of the Year." He was also a member of the American Society for Animal Production, which he served as vice-president of the North Atlantic Section and later was its chairman. To achieve closer integration of the dairy industry in Vermont, he organized the Vermont Dairy Plant Operators' and Managers' Association and was its executive secretary for 20 years. When he resigned as secretary in 1941, the association presented him with a citation embossed in gold letters and enclosed in a gold frame and made him honorary secretary. His good judgment and integrity were recognized by state and industry people in 1931, when he was selected as chairman of the New England Governors' Dairy Advisory Board to organize and administer the Boston market milk area for 18 months.

Dr. Ellenberger is versatile in what may be called hobby activities. He is an excellent photographer, is at home in the carpentry shop, and can raise flowers that would be a credit to professionals. At present he is living in Orlando, Florida. His contributions to the dairy industry have won for him the recognition of being one of its great pioneers.

E. O. HERREID

The June Issue

On July 17, 1906, the first meeting of an organization that later was to be called the American Dairy Science Association was held on the campus of the University of Illinois at Urbana. To commemorate this event and the 50 years of industry progress since then, a special issue of the Journal will be printed in June. This anniversary issue will be considerably

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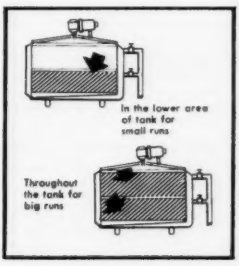

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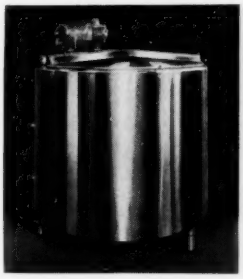

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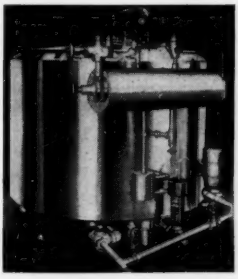

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larger than regular numbers and will contain special articles written on invitation of the editor by persons considered authorities in their respective fields. These articles will record the progress made in the sciences basic to research in dairying and the developments in the various branches of the industry since 1906. Because of the probable demand for copies, arrangements are being made to have an extra supply available for purchase.

Statistics from the Secretary-Treasurer's Desk

Classification of 1956 Membership in the American Dairy Science Association[1]

	Number	*%*
Teaching, research or extension	738	42.30
Commercial dairy plants (Mfg.)	572	33.00
Dairy equipment & supplies mfg. or sales	91	5.20
Private laboratories or consulting	60	3.40
Farming	49	2.74
Breeding associations	48	2.69
State departments of agriculture	27	1.60
Trade associations	26	1.40
Feed manufacturing	22	1.26
Milk producers associations	21	1.10
Health departments	13	0.75
Retired or unemployed	13	0.75
Breed associations	12	0.66
Army	12	0.66
Publishing	11	0.63
Veterinarians	11	0.63
County agents or farm advisors	8	0.50
Cooperative farm supplies	5	0.30
Navy	4	0.20
Milk market administrators	2	0.12
Housewife	1	0.05

[1] The dues notices for 1956 asked: (1) By whom employed and (2) Type or title of position. As of April 1, 1,746 replies had been received out of a membership of approximately 1,950. It is believed that the ones missing would have little, if any, effect on the percentage figures.

Memberships, Affiliate Memberships, and Subscription Status

April 1, 1956

	12/31/54	*12/31/55*	*4/1/56*
Members	1,787	1,775	1,963
Affiliate Members	362	409	468
Subscriptions	1,454	1,421	1,430

Complete Journal File Available

A complete set of American Dairy Science Association Journals, starting with Volume 1, which belonged to the late H. C. LINDQUIST of Massachusetts, is available. Interested parties should contact Mrs. Lindquist at 47 Fearing St., Amherst, Mass.

Minnesota Dairy Industry Career Day

The "Minnesota Dairy Industry Career Day" was held on the St. Paul campus of the Univ. of Minnesota on March 17. Present were between 335 and 350 young men and women, together with their parents, high school advisors, and men of the industry.

This large attendance was largely due to the fine cooperation of the industry and its friends throughout the state. After a tour of the facilities of the dairy department the group assembled in the auditorium of Coffey Hall. One feature of the program was a report by alumni majors in the field of dairy industry, who took as their subject, "My Career in the Dairy Industry." In addition, talks were given by A. A. DOWELL, Director of Resident Instruction, KEITH MCFARLAND, Asst. to Director of Resident Instruction, and H. A. MORRIS of the Dairy Department.

A buffet luncheon, sponsored by the Minnesota Dairy Industry Committee with the assistance of faculty and graduate students of the Dairy Department, was served the group.

Cornell Holds Symposium on Enzymes

A symposium on Bacterial Enzymes, sponsored by the Department of Dairy Industry and the Laboratory of Bacteriology was held April 12 at Cornell Univ. Highlights of the symposium were two lectures presented by scientists from France. DR. ANNA MARIA TORRIANI of the Pasteur Institute, Paris, discussed the induced biosynthesis of bacterial enzymes, including penicillinase, and DR. LUIGI GORINI of the Sorbonne, Paris, spoke on bacterial proteinase and specific conditions necessary for activity.

Ohio State Holds Career Day

Approximately 200 high school students, principals, and counselors and industry representatives from Ohio and northern Kentucky attended the third annual Dairy Technology Career Day at Ohio State Univ. on April 14. This event constitutes the climax of the year's high school counseling and recruitment program conducted cooperatively by the organized dairy industry groups of Ohio and the Department of Dairy Technology.

The morning program opened with conducted tours, at which time research and dairy plant operations were demonstrated and explained. This was followed by a welcome address by I. A. GOULD, chairman of the Department, who briefly outlined the Dairy Technology scholarship program and curriculum to the prospective college students.

Personal experiences encountered in the transition from high school to college and student club activities were related by a senior, a junior, a sophomore, and a freshman in dairy technology. Showing of the film, "The Ohio State University Story," completed the morning program.

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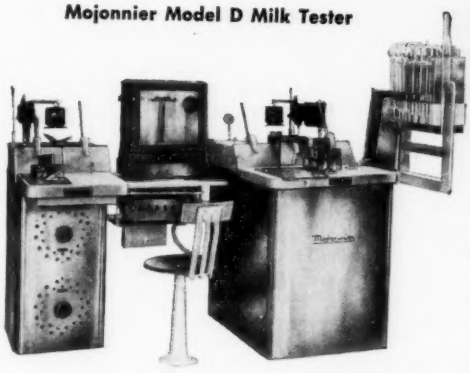

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At the luncheon C. W. HUTCHISON, assistant dean of the College of Agriculture, welcomed the students to the campus. The cartoon slide sequence, "Opportunities Unlimited," depicted the advantages and career opportunities in the dairy industry field. F. E. GREINER, executive secretary of the Ohio Dairy Products Association, narrated the cartoon. "Opportunities Unlimited" was produced and financed by Ohio Dairy Boosters Assoc. and presented to Ohio Dairy Products Assoc. for distribution.

As a major part of the Ohio Dairy Technology high school counseling and recruitment program a series of six dinner meetings were held throughout Ohio for high school principals and counselors, students, and parents. These meetings were sponsored and financed by local dairy organizations. The purpose of the meetings was to inform high school principals and counselors about the pre-college background most suitable for the curriculum in dairy technology, the scholarships available to incoming students and the professional opportunities for graduating seniors.

The scholarship program at Ohio State includes about 15 scholarships available to freshmen entering the field of dairy technology. Of these, four are of the sustaining type, and the remainder are $300 scholarships designed to attract freshmen to the field. The recipients are selected on the basis of their high school record, by personal interviews with representatives of the commercial industry and the Department of Dairy Technology, and by written examinations in the fields of science, mathematics, and English.

The scholarship program is sponsored by various dairy technology societies, dairy companies, and local dairy organizations in Ohio.

DALE SEIBERLING, Department of Dairy Technology, spoke on "Engineering for Plant Safety" before the Dairy Section of the Annual All-Ohio Safety Congress April 17 in Cincinnati.

C. J. BLACKBURN (M.S. Iowa State '55) has been employed by Fairmont Foods, Columbus, as an industrial engineer.

A Speakers Bureau has been established by the Ohio Dairy Products Assoc. for the June Dairy Month festivities. The speakers will be available on request.

C. A. HOULETTE, Columbiana, Ohio, has been elected assistant secretary-treasurer of the National A.D.A.

American Dairy Exhibit at Japanese Trade Fair

An American Dairy Princess and liberal samples of American dairy products proved equally attractive to thousands of Japanese who visited the International Trade Fair, Osaka, April 8-22. The booth was a cooperative effort of Dairy Industries Society, International (as an educational effort in its market development program); U. S. Departments of Agriculture and Commerce; American Dairy Association; and National Cheese Institute. The effort also had the generous assistance of numerous members of the Japanese dairy industry.

The American Dairy Princess, RUTH MARIE PETERSON of Minnesota, assisted American Ambassador JOHN M. ALLISON in opening the dairy booth exhibit, installed and operated by DISI. From the booth were distributed samples of ice cream and fluid milk made from dry components shipped from the United States, as well as cheese. The ice cream and milk were processed by American machinery before the eyes of the audience.

Educational material to interest Japanese visitors in health values of milk in the diet, and industry conferences for the benefit of milk-industry people, were added features.

The American Pavilion at the Osaka Fair was part of the International Trade Fair program of the United States government. Food industry exhibits were financed with local currencies available under Public Law 480 (derived from sale of American agricultural surpluses abroad) to "help develop new markets for U. S. agricultural commodities on a mutually benefiting basis." United States Department of Agriculture also provided to the DISI exhibit specialists 36,000 lb. of nonfat dry milk solids and 15,000 lb. of anhydrous butterfat for milk recombining and soft-serve ice cream making. Cheese was provided by the (U. S.) National Cheese Institute.

This was the second time DISI has cooperated with U. S. government agencies in staging a dairy exhibit at an International Trade Fair. The first cooperative dairy exhibit was operated by the Society at the Bogotá, Colombia International Trade Fair, Nov. 25–Dec. 11, 1955. Each time the United States dairy industry through DISI contributed or loaned substantially all the equipment and supplies used and supplied the person or persons to set up "a small milk plant and ice cream factory" at the exhibit, consult on industry problems locally, and supervise educational efforts.

These tasks were ably handled at the Osaka Trade Fair by IRVING C. REYNOLDS. Long known internationally as a "Dairy Ambassador" for his volunteer dairy industry building efforts, ranging from many areas of the Far East to the Caribbean area, Mr. Reynolds is a past president of DISI and of the National Assoc. of Retail Ice Cream Manufacturers, Inc.

Eli Lilly Expands Research Facilities

A $3,000,000 expansion program in agricultural research was announced recently by Eli Lilly and Company, of Indianapolis, who will construct an agricultural research center on 417 acres at Greenfield, Ind., present site of the Lilly Biological Laboratories.

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Lilly veterinary research will be closely coordinated with activities of Corn States Laboratories, Inc., of Omaha, a recently purchased subsidiary.

McCoy Advances in Pennsalt's Technical Division

GEORGE MCCOY has been named manager of the Pennsylvania Salt Manufacturing Company's Research & Development Department, a major component of Pennsalt's recently created Technical Division. Other units of this division, which is directed by Vice-President W. A. LALANDE, are the Central Engineering, Patent, Commercial Development, and Sales Services Departments.

McCoy attended the Univ. of Pennsylvania, where he earned the bachelor's degree in 1939 and the doctorate in 1943. Immediately before joining Pennsalt in 1944, he was an instructor in organic chemistry at Penn State. During his association with the company, he served successively as senior research chemist, group leader, assistant director, and director of organic research.

Lederle Supports Dairy Industry Research

The Lederle Laboratories Division of the American Cyanamid Co. has renewed, for another 18-month period, a grant to the Cornell Univ. Dairy Department for support of research on microbial enzymes and their industrial uses. F. V. KOSIKOWSKI is directing this project.

Diversey Publishes Technical Bulletin on Psychrophilic Bacteria

An 8-page brochure, analyzing off-flavor problems caused by psychrophilic bacteria, is available from The Diversey Corp. of Chicago. Titled, "Control of Off-flavors in Pasteurized Milk and Slime on Cottage Cheese Due to Psychrophilic Bacteria," this technical bulletin explains what psychrophiles are, their growth habits, their effect on product quality, and how to control them.

Milk Hauling Costs

Service Report 18 on "Farm-to-Plant Bulk and Can Milk Hauling Costs" by JOSEPH M. COWDEN of the Farmer Cooperative Service, USDA, has just been released. The Farmer Cooperative Service conducts research studies and service activities in connection with cooperatives engaged in marketing farm products, purchasing farm supplies, and supplying business services. Copies of Report 18 can be obtained from JOSEPH G. KNAPP, Administrator of the Farmer Cooperative Service.

Completed Thesis

Ph.D. Degree:

EMMETT W. BASSETT—Isolation and identification of acidic and neutral carbonyl compounds in various cheese varieties. The Ohio State Univ., Columbus.

Impromptu Speaking

It is sometimes necessary to speak extemporaneously. A simple rule that will help organize your thoughts in a logical manner may save the day. One of the following four apporaches may be used.

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   The future
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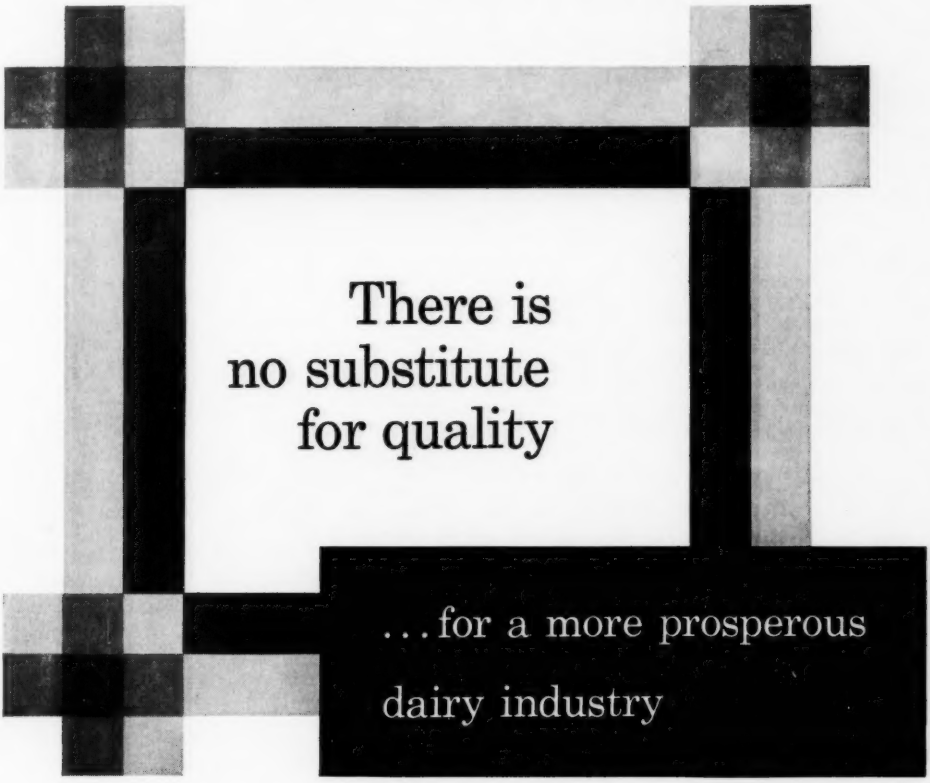
   The cause
   The solution
3. The advantages
   The disadvantages
   The solution
4. The need
   The fulfillment
   The action necessary



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STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

Cornell Students Sponsor Centennial Exhibit

A centennial celebration of the establishment of the first butter factory in the United States was sponsored by the Cornell Dairy Science Assoc. during Farm and Home Week March 19-23. Under the supervision of E. S. GUTHRIE and R. P. MARCH, a replica was built of the original factory established at Campbell Hall, New York, in 1856 by W. R. WOODHULL. The exhibit included century-old equipment used in buttermaking and pictures of the original site. The replica will be exhibited again at the New York State Fair in September.

Florida Chapter Honors Dairy Department Staff

The annual Dairy Science Club meeting in honor of the Dairy Science Department's staff, the workers, and their families was held in the form of a Bar-B-Que on March 17 at the Univ. of Florida Dairy Research Unit. Over 100 persons attended. Besides the traditional barbecue, slaw, and beans, the menu consisted of dairy products and home-made cakes. Credit for preparation of the food went to DOUG BOYETTE.

The evening was complemented by participation of Univ. of Florida students who generously contributed to the entertainment. The program, under the direction of WM. HARROD, opened with a series of western songs, continued with harmonica and piano playing, and ended with a novelty of hill-billy tunes.

Louisiana Dairy Science Club Activities

The student affiliate membership drive in the LSU Dairy Science Club was particularly successful this year. Sixteen new members were added, bringing the total membership to 29.

The club sponsored a lecture and discussion by DR. JOHN B. ENTRIKIN on the topic "Science and Religion" on Feb. 29. This is the first time that a departmental club has sponsored a Religious Emphasis Week speaker on this campus. The experiment was so successful that the club plans to add this to its regular schedule of programs.

The 1956 LSU Dairy Cattle Judging Team got off to a good start at the Fort Worth Fat Stock Show held Jan. 26. The team tied for first place with Arkansas, and the president of the Dairy Science Club was high man in judging Holsteins. The trip was sponsored jointly by the Dairy Department and the Dairy Science Club.

M.S.U. Dairy Club Winter Quarter Events

Activities by the Michigan State Univ. Dairy Club during the winter quarter, 1956, included the Fitting and Showing Contest (won by ROBERT JOHNSON), a square dance with the Dairy Department staff as guests, and the annual Agriculture Honors Banquet.

The Ag Honors Banquet is sponsored by the Agriculture Council, which consists of representatives from the various clubs in the College of Agriculture. Awards were made to members of the judging team. JOHN HOSTETLER, retiring president of both the Dairy Club and the Ag Council, was presented the Activities Award. DAVID BOYNE, former Dairy Club representative to Ag Council and newly elected Council president, was general chairman for the Banquet.

Montana Club Meets with Faculty

On Feb. 16, the Montana State Dairy Science Club sponsored a dairy staff-club waffle feed. After the supper, the group was entertained by DON GILL, who showed his collection of slides on Iraq.

Penn. State Club to Sponsor Dairy Exposition

This year the Penn. State Dairy Science Club sponsored the 31st annual Dairy Exposition. The show, a feature of the Mother's Day weekend, was held May 12. This year's show honored R. H. RUMLER, executive secretary of the Holstein-Friesian Assoc. of America.

DARWIN BRAUND, show manager, assisted by NORMAN SCHUE, handled the over-all details of arranging the annual program, which includes dairy products judging contests, fitting and showing competition, and dairy cattle judging. Heading the preparation in dairy manufacturing was BRUCE KNAUSS. The program included contests in milk and ice cream judging for both amateurs and professionals.

Animals from all five purebred dairy herds of the University were drawn by entries in the fitting and showing competition. Judging the fitting and showing contests was G. M. CAIRNS, dean of the College of Agriculture, Univ. of Maryland. Aiding him was C. E. HARDING, of Maplevale Farm, Brookville, a Penn State graduate in 1950.

Tennessee Dairy Club News

The Tennessee Dairy Products Assoc. held its annual meeting in Knoxville, Feb. 13-14. A highlight of the meeting was an invitation to

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Admitting promptly and freely that no compound of this sort is an ideal container for milk, it is still entirely possible with not too much work to keep the Surge Black Inflation so clean that no contamination can be traced to it.

This is an advertisement, but even so we are saying exactly what we sincerely believe to be true. We most certainly would neither build nor sell this Surge Black Inflation if experience did not satisfy us that it is the most satisfactory article now being built.

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all dairy students to be present as guests of the Association at its luncheon session. The speaker was BUFORD ELLINGTON, Tennessee Commissioner of Agriculture. On Feb. 20-21, the annual Tennessee Dairy Institute was held on the campus. The Club served the noon meals.

The Club ended the winter quarter activities with a talk by ROYAL SHANKS from the Botany Department, who spent last summer in Alaska beyond the Arctic studying the vegetative life of the area.

Babcock Dairy Science Club Activities

Getting acquainted with the ADA was the subject of an interesting and up-to-date talk by MR. DICKENSON, speaker at the March meeting of the Babcock Dairy Science Club. He told of the problems facing the dairy industry today, and discussed the activities of the ADA with respect to sales promotion, methods of operation, financing, and results obtained.

Dairy people from all over the state attended the annual Dairy Manufacturers' Conference held March 13 and 14 at Babcock Hall. Speakers from all phases of the dairy industry took part in the program. During the intermission between each session of the Conference, club members sold refreshments. The income from this activity will be used to help sponsor the Dairy Products Judging Team.

A Reminder

News items for the Student Chapter News Section should be sent to: E. L. THOMAS, Dept. of Dairy Husbandry, Univ. of Minnesota, St. Paul 1. These must be received by the 10th of each month in order to be published in the following month's issue of the Journal.

Brief articles (200 words) on the history and special activities of the various student branches are welcomed. Photographs of general interest will be considered for publication when space permits.

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LETTERS TO THE EDITOR

17th April, 1956.

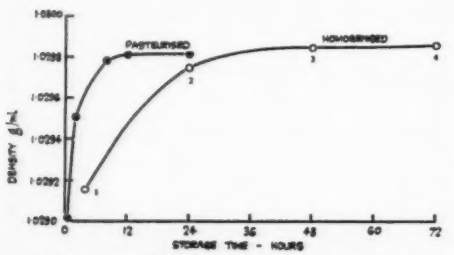
Dear Sir,

Some Physical Properties of Milk: 1. Density

The work reported by Rutz, Whitnah and Baetz in the December 1955 issue of Journal of Dairy Science is similar to work which I have recently completed at the National Institute for Research in Dairying, Reading, England. There are some criticisms of the work of Rutz *et al.* that I should like to make.

Rutz assumes that the Reeknagel effect in homogenised milks is completed in a similar period to that for raw or pasteurised milks, namely 18 hours. It has been found that in homogenised milks, a period of 48 hours' storage is required to bring about the complete contraction *(1)*. Also, contrary to Rutz, I have found that homogenisation at a pressure of 3000 lbs/sq. in. increases the density of milk.

In a study of the Reeknagel contraction in homogenised milks, samples of the milk were stored at 2-3° C. The density measurements were made at 20° C. after rapidly warming from storage temperature. The homogenisation was carried out under 3000 lbs/sq. in. and at 145° F. The results are shown in the figure



THE DENSITY OF PASTEURISED AND HOMOGENISED MILK DURING COLD STORAGE.

for the density of four samples of homogenised milk, 1, 2, 3 and 4, which were stored for periods of 4 hrs., 24 hrs., 48 hrs. and 72 hrs. respectively. A comparative curve of the results for pasteurised milk is also shown. It may be seen from the figure that the contraction in homogenised milk is only 75% completed after 18 hours. The densities of homogenised milk samples which have been stored for varying periods less than 48 hours cannot be compared, therefore, since the milk fat will not be fully contracted. To obtain the effect of homogenisation on the density of milk, the densities of homogenised and pasteurised samples were compared. A pressure of 3000 lbs/sq. in. and a temperature of 145° F. were used in the homogenisation. To ensure that the milk fat was in the same physical state in both the pasteurised and the homogenised milks, the samples of milk were prewarmed to 45° C. for 1 minute and the density measurements made at 20° C. The density of the homogenised milk was 6.5×10^5 g/ml greater than that of pasteurised milk for samples containing approximately 3% fat and 10×10^5 g/ml greater for samples containing 6% fat.

Pyenson and Dahle *(2)* have shown that efficient homogenisation increased the bound water content of milk whilst less efficient homogenisation decreased it. This might explain the effect of homogenisation on the density of milk since increases in bound water content in a sample increase the density of the sample.

In view of the uncertainty of the physical state of the milk fat in samples of homogenised milk used by Rutz *et al.*, the coefficient of density with temperature quoted (0.00038 g/ml/° C) in their paper has also an element of uncertainty and is probably too high. The Sharp and Hart *(3)* modification of the Quevenne Lactometer procedure is designed so that the fat in the milk under test is in the liquid state. The coefficient I have obtained *(4)* for prewarmed milk of composition 4.0% fat and 9.0% S.N.F. is 0.00033 g/ml/° C. This coefficient is applicable for the temperature range 10° to 32.5° C. The relationship between density and temperature is linear within the limit of $\pm 1 \times 10^{-4}$ g/ml for the temperature range 10° to 30° C. for milks containing over 3% fat and the linearity extends above 30° C. with increasing fat content, the range being 10° to 38° C. for a milk containing 5.0% fat and 9.0% S.N.F. Rutz has assumed linearity for a milk containing 4.0% fat and 9.0% S.N.F. for the temperature range 18° C. to 44° C. The departure from linearity of the density/temperature relationship for such a milk is 12×10^{-4} g/ml at 44° C.

The coefficient varies 0.000,008 per 0.1% S.N.F. and 0.000,017 per 1% fat. A coefficient of 0.000,038 should only be obtained for a milk containing 6.0% fat and 9.25% S.N.F.

I am indebted to Professor Rutz for discussions on these matters, by correspondence.

A. L. Short

The National Institute for Research in Dairying Reading, England

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(1) Walker, H. *Analyst*, 70: 372. 1945.
(2) Pyenson, H., and Dahle, C. D. *J. Dairy Sci.*, 21: 407. 1938.
(3) Sharp, P. F., and Hart, R. G. *J. Dairy Sci.*, 19: 683. 1936.
(4) Short, A. L. *J. Dairy Research*, 22: 69. 1955.

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March 5, 1956

To the Members of the Dairy Industry:

Since writing to you in January I have traveled in Germany and Austria and visited dairy laboratories, dairy plants, dairy farms, and experiment stations. Everywhere the people treated me with the utmost courtesy, and I have never had more interested listeners than those who attended the 13 lectures that were scheduled. The lectures had been well advertised, and select groups of agricultural and dairy people had been invited to attend. So far as I know, it was the first time the United States Foreign Service had scheduled lectures on the dairy industry by an American.

The meetings were held either at the "Amerika Haus" or in an auditorium in a college or at an experiment station. I first showed 12 USDA color slides of the present status of the dairy industry in the United States. I then talked for about 50 minutes about the economics of the dairy industry, developments in dairy farming and milk handling methods, and developments in the manufacture and merchandising of dairy foods. I mentioned the ADA and state programs to promote the use of dairy foods. I used illustrations. I then showed the sound color movie: "America's Favorite," the National Dairy Council's film on ice cream. Then there were many questions, and some meetings lasted over 2 hours.

The lectures were held in, or in the vicinity of, Kiel, Hamburg, Hannover, Kaiserslautern, Stuttgart, München, Regensburg, Landshut, Kempten, Vienna, and Linz. I visited the large dairy research stations at Kiel, Hohenheim, and Weihenstephan and dairy plants in the vicinity of Kiel, Hamburg, and Ludwigshafen, as well as dairy farm experiment stations at Kiel, Neumühle, Hohenheim, Landshut, and Weihenstephan. I took 200 photographs.

One morning at 7:00 I left Regensburg by train for München (Munich), the old Nazi stronghold. I gave a lecture in a former Nazi building that afternoon, then left by car at 5:00 p.m. for Kempten, about 100 miles away in the foothills of the Alps. I noticed one highway sign that pointed to Dachau. Later, one pointed to Oberammergau. One of these names is infamous; the other is the name of the town where each 10 years the Passion Play is held. (Next in 1960). The road was winding and it was raining. We arrived at the college at 8:15 and the auditorium was full of people. The meeting lasted until 11:00, and we drove back to Munich through a snowstorm, arriving at 2:00 A.M.

My last appearances were in an old castle in western Austria. Two annual meetings of Austrian dairy associations were held there with about 50-60 in attendance each day. Many were leading farmers. It was a pleasure to speak before these groups, who listened carefully. Many wore the typical Austrian clothes. The chairman expressed gratitude in the charming and sincere Austrian way.

Here I have had the opportunity to express before these audiences how much better life is in the United States and in the free world on account of the contributions by great Austrian scientists, such as Mendel, and by the many composers and artists. I mentioned Mozart, who was born 200 years ago in nearby Salzburg, and Wagner, Strauss, Haydn, and Bruckner. I closed my last lecture by assuring the Austrians of the continuous friendship of the American people.

You may have heard that the weather has been cold in Europe. It is true. All during February we had freezing weather, with temperatures down to zero. The waters surrounding Denmark, except on the west coast, froze over. Recently I went by ship to the Island of Bornholm in the Baltic Sea south of Sweden. Ice 6-10 in. thick covered the water all the way, and once an ice-breaker had to help us get free. On account of a heavy snowfall the roads on the Island had been closed and the dairy meeting I was to attend had been canceled. There are about 10 creameries on Bornholm. A large cooperative plant manufactures 2 million pounds of "St. Clemens" brand blue cheese per year. Of this, 85% is exported to Great Britain, French Morocco, Germany, Japan, United States, Egypt, Italy, Switzerland, Sweden, South America, and Iraq. The cheese is of excellent quality.

The University of Nottingham, England, has invited me to give a series of lectures, which will be under the auspices of the United States Educational Commission in the United Kingdom. It will be an interesting experience.

G. H. WILSTER
Copenhagen, Denmark

OUR ASSOCIATION

The Challenge of Education

DEAR FELLOW MEMBERS:

This year, our Association has once again turned its attention to the subject of "dairy education" and "instruction." At the last annual meeting, the Executive Board created an Education Committee and this committee, in turn, has made plans for a session on dairy education at the forthcoming meeting in June. The question now is, "Will this interest in the important subject of classroom teaching be maintained in subsequent years, or will it deteriorate and disappear as it has in the past?"

Let us look to our past in this connection.

Our Association was organized in 1906 basically for the purpose of improving teaching and disseminating technical information. However, over the years, the major attention in the Journal and at the annual meeting has been given to research—with the result that the educational objective of the Association as it relates to undergraduate and graduate instruction has tended to be relegated to the background. Periodic attempts have been made, usually at the annual meetings, to direct attention to the area of instruction. Committees have been appointed and programs developed, but these spurts of organized interest were short-lived—lasting only a year or two—at any one time. This may be taken as evidence that the officers and program committees of the Association were not convinced of the need for a continuing program in this area.

What has happened as a result of this neglect?

First: Our Association has lost pace with professional organizations in other fields which, years ago, established ambitious and continuing programs—and even sections—on education.

Second: We have failed to serve our membership adequately and fully. The technical sessions at the annual meetings are of vital concern to the research man but of less concern, in total, to the University staff member or administrator whose responsibility lies largely in instruction. Such a person could profit greatly by discussions on such subjects as *(a)* a critical review of curricula, *(b)* evaluation of staff members as teachers, *(c)* using teaching aids effectively, *(d)* objectives of a University education in dairying, and *(e)* integrating classroom and extra-curricular activities. By such meetings the teachers could share views on how to do a better job in University teaching and, in this manner, be encouraged to strive constantly for improvement through self and group appraisal.

Third: We have failed to assume and maintain the leadership in dairy education expected of us by the commercial industry. Attention has been focused on this fact in recent years as a result of the shortage of University-trained men, particularly in the dairy manufacturing industries. Editorials have appeared in trade periodicals criticizing dairy curricula, teaching methods, and lack of cooperation between University staff and commercial interests. Of particular note is the fact that the Association was criticized specifically for not publicly recognizing the needs of the commercial industry and for not encouraging the dairy educators to make a critical appraisal of their own curricula and teaching and to take appropriate action to get "their houses in order."

In recent years, the annual meetings of national dairy trade associations have featured discussions on such subjects as "What the college-trained man expects of industry" and "What the commercial dairy industry expects of a college-trained man." Our Association has been essentially silent. National surveys have been made of the college manpower situation in dairy manufacturing. Our Association has not been involved officially. These developments should give us cause for concern.

Fourth: By neglect of our educational responsibilities as an Association we penalized our own graduates. The Resident Instruction Section of the American Association of Land-Grant Colleges and Universities passed a resolution at its 69th annual convention in 1955 which included the following statements:

> "One place where the teacher may be stimulated to a better (teaching) performance is in his professional society . . . Most of those entering Land-Grant Colleges from graduate schools will be given full- or part-time teaching jobs, (yet) there is a marked tendency to give consideration only to preparing the student to do research. . . . Even where a student expects to devote his efforts entirely to research, many of the characteristics that make for a good teacher are invaluable to the full-time research worker. . . . *In some professional societies, there is relatively little place for the teacher* (and) *the sections and programs tend to ignore him.* The Resident Instruction Section urges (the professional societies) to give careful attention . . . to the (development) of good instructors."

As members of a professional organization, we acknowledge the fact that good teaching produces good graduates—persons who are qualified to use their inherited abilities to the fullest. Furthermore, good teaching requires a diligence, a devotion to duty, and a creativeness equal to the requirements for research.

Last year, the Association made its first award for outstanding teaching in dairy manufacturing, sponsored by the Milk Industry Foundation and to be given in alternate years. Recently, the Executive Board accepted the offer of the National Dairy Products Company

to make a similar award of $1,000 and a bronze plaque for an outstanding teacher in dairy production. The first award of this series will be at the June meeting, and subsequent awards will be made available in alternate years thereafter. These awards indicate that the Association is turning its attention in larger measure to its responsibilities in teaching. Perhaps these recognitions will insure the continued interest of the Association in this subject.

It is not the intent to outline here a future program for the Association in the realm of dairy education. However, this much may be said: The gauntlet has been thrown down—and the challenge placed squarely in our path. There seems to be no alternative but for the Association to create a permanent Education Committee and charge it to press forward vigorously to reestablish our Association as a strong, progressive, educational force for our industry. If such a committee is to discharge its full responsibilities, it will need leadership with initiative, vision, understanding, and perseverance. The task that needs to be accomplished is not an easy one.

As a concluding thought in respect to the educational work of our Association, we need give full credit to the Extension Section, which has long given major attention at the annual meetings to extension teaching. The same principles apply to classroom teaching, and our Association as a whole can well profit from the Extension Section's experiences.

Odds and Ends

Recent report from Secretary-Treasurer Judkins indicates a favorable financial picture for the year and with the membership close to the 2,000 level established as a goal last July. It may be reached by June. Of the membership, 42% are in teaching, research, and extension and 33% are from commercial dairy plants. Credit for the membership increase rightfully belongs to the Membership Committee and to Lyman Rich, its chairman. The large number of members from the commercial manufacturing field emphasizes the need for having a program which will serve this segment adequately.

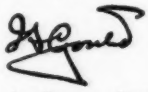
The June issue of the Journal will be approximately three times the usual size and will be an outstanding historical document. All of the articles in this number will have been prepared especially for it, and the regular sections of the Journal will be withheld for subsequent issues. A feature of the June issue will be an unusually fine history of our Association by Historian G. M. Trout, Michigan.

The new format of the Journal, introduced in March, came about after great deliberation by the Journal Management Committee. Additional changes are contemplated. Chairman J. H. Erb and Editor Tracy will welcome your views in regard to these changes—some of which are being introduced on a temporary basis. The changes are designed to make the Journal more acceptable to its widely diverse reading public and, also, to permit more effective display of advertising. We must remember that advertising is an important means of financing the Journal's costs.

The Golden Jubilee Meeting in June will honor the six living charter members of our Association at the opening session: C. F. Doane, Oregon; E. S. Guthrie, New York; C. C. Hayden, Ohio; O. F. Hunziker, Illinois; C. E. Lee, Wisconsin; and C. E. Thom, New York. During this year, the Executive Board awarded special charter honorary memberships to Messrs. Doane, Thom, and Lee. The other living charter members had been awarded honorary memberships earlier.

Of particular significance at the annual meeting will be a new approach to be used at the Second General Session (previously designated as the Business Session) to make this feature of more general interest. A full attendance of the members will help to assure the success of this innovation.

Cordially yours,



I. A. GOULD, *President*

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PROGRAM

FIFTY-FIRST ANNUAL MEETING

of the

AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF CONNECTICUT

STORRS, CONNECTICUT

June 18-21, 1956

Program Committee

H. D. EATON, Connecticut
W. M. ROBERTS, North Carolina
N. P. RALSTON, Michigan
G. M. WERNER, Wisconsin
R. E. ERB, Washington, *Chairman*

GENERAL PROGRAM

Sunday, June 17

9:00 A.M. **Meeting of the Executive Board,** *Wood Hall Lounge*

Monday, June 18

9:00 A.M. **Meeting of the Executive Board,** *Wood Hall Lounge*

10:00 A.M. **Registration,** *Old Basketball Cage (ROTC)*

1:00–5:00 P.M. **Campus Tours,** *Assemble in New Haven Hall Lounge, North Campus*

5:30 P.M. **Past Presidents' Dinner,** *Grill—North Room*

8:00 P.M. **Open House,** *Student Union*

Tuesday, June 19

8:00 A.M. **Registration,** *Old Basketball Cage (ROTC)*

9:30 A.M. OPENING SESSION, *University Auditorium*

DR. W. A. COWAN, *Head, Animal Industries Department, University of Connecticut, presiding.*

Prelude
DR. WALTER IHRKE, *Head, Music Department, University of Connecticut.*

National Anthem
MR. ROY E. NORCROSS, *County Agent, New Haven County, Connecticut.*

Invocation
REVEREND J. GARLAND WAGGONER, *Minister, The Storrs Congregational Church.*

Welcome
DR. W. B. YOUNG, *Dean and Director, College of Agriculture, University of Connecticut.*

Golden Jubilee Ceremony
DR. IRA A. GOULD, *Chairman, Department of Dairy Technology, Ohio State University.*

Presidential Address
DR. IRA A. GOULD

Address
DR. A. N. JORGENSON, *President, University of Connecticut.*

Postlude
DR. WALTER IHRKE

SECTION MEETINGS

1:30–3:30 P.M. MANUFACTURING SECTION A
Dairy Chemistry
Student Union Ballroom

3:30–4:30 P.M. MANUFACTURING SECTION BUSINESS MEETING
Student Union Ballroom

1:15–5:00 P.M. PRODUCTION SECTION A
Reproduction and Artificial Insemination
Engineering 207

1:15–5:00 P.M. PRODUCTION SECTION B
Milk Secretion
Ratcliffe Hicks Livestock Arena

1:15–3:15 P.M. EDUCATION COMMITTEE (JOINT MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)
Hawley Armory
Selected Topic: Educational Objectives in Dairy Science
E. L. JACK, *University of California, Davis, Chairman*

For Dairy Manufacturing Students
H. B. HENDERSON, *University of Georiga*

For Dairy Production Students
W. E. PETERSEN, *University of Minnesota*

Film "Accent on Learning"
T. D. HARMAN, *Ohio State University, Columbus*

1:30–3:30 P.M. EXTENSION SECTION

Opening Business Session and Dairy Records Committee Reports
College of Agriculture Auditorium

3:40–5:00 P.M. EXTENSION SECTION

Presentation and Discussion of Exhibits
College of Agriculture Auditorium

8:00 P.M. **Square and Round Dancing**
Hawley Armory

Wednesday, June 20

SECTION MEETINGS

8:30–10:30 A.M. MANUFACTURING SECTION A (JOINT WITH PRODUCTION AND EXTENSION SECTIONS)

Selected Topic: Control of Off-Flavors in Milk
Student Union Ballroom

8:30–10:45 A.M. MANUFACTURING SECTION B

Dairy Chemistry
Engineering 207

11:00 A.M.–NOON MANUFACTURING SECTION BUSINESS MEETING
Student Union Ballroom

8:00–11:00 A.M. PRODUCTION SECTION A

Calf Nutrition
College of Agriculture Auditorium

8:00–11:00 A.M. PRODUCTION SECTION B

Genetics
Ratcliffe Hicks Livestock Arena

11:00 A.M.–NOON PRODUCTION SECTION BUSINESS MEETING
College of Agriculture Auditorium

8:30–10:45 A.M. EXTENSION SECTION
4-H Program
Ratcliffe Hicks 10 and 11

11:00 A.M.–NOON EXTENSION SECTION BUSINESS MEETING
Ratcliffe Hicks 10 and 11

1:30–4:30 P.M. MANUFACTURING SECTION A
Symposium: Special Dairy Products
Student Union Ballroom

1:30–4:00 P.M. MANUFACTURING SECTION B
Dairy Bacteriology
Engineering 207

1:15–3:15 P.M. PRODUCTION SECTION A
Ketosis and General Physiology
College of Agriculture Auditorium

1:15–3:15 P.M. PRODUCTION SECTION B
Pasture and General Nutrition
Ratcliffe Hicks Livestock Arena

1:15–3:15 P.M. EXTENSION SECTION
Parasite Control in Dairy Cattle and
Special Quality Milk Program to Control Flavors
Ratcliffe Hicks 10 and 11

3:30–4:30 P.M. PRODUCTION AND EXTENSION SECTIONS
Joint Committee Reports
Ratcliffe Hicks Livestock Arena

3:15–5:15 P.M. STUDENT AFFILIATE AND FACULTY ADVISOR MEETING
W. L. SLATTER, *Chairman*
Discussion of Problems and Plans for a More Comprehensive Student Affiliate Program
Student Union 101 and 102

4:30–5:30 P.M. EXTENSION SECTION
MEETING—SUPERINTENDENTS OF OFFICIAL TESTING
Ratcliffe Hicks Livestock Arena

Thursday, June 21

SECTION MEETINGS

8:30–10:00 A.M. MANUFACTURING SECTION A
Milk
Student Union Ballroom

8:00–10:00 A.M. PRODUCTION SECTION A
Nutrition and Silage
College of Agriculture Auditorium

8:30–9:50 A.M. EXTENSION SECTION
Sire Evaluation and Management Factors Influencing Yields
Ratcliffe Hicks Livestock Arena

10:00 A.M.–NOON GENERAL SESSION DEVOTED TO ASSOCIATION AFFAIRS
University Auditorium

1:30–4:30 P.M. MANUFACTURING SECTION A
Dairy Products
Student Union Ballroom

1:15–4:30 P.M. PRODUCTION SECTION A
Ruminant Nutrition
College of Agriculture Auditorium

1:15–1:45 P.M. EXTENSION SECTION
Management
Ratcliffe Hicks Livestock Arena

2:00–4:00 P.M. EXTENSION SECTION (JOINT WITH MANUFACTURING AND PRODUCTION SECTIONS)
Selected Topic: Aspects of Mastitis Control
Ratcliffe Hicks Livestock Arena

8:00 P.M. RECOGNITION PROGRAM
University Auditorium

ENTERTAINMENT

GENERAL PROGRAM

Monday, June 18	8:00 P.M.—**Open House** *Student Union*
Tuesday, June 19	8:00 P.M.—**Square and Round Dancing** *Hawley Armory*
Wednesday, June 20	**No Entertainment**
Thursday, June 21	8:00 P.M.—**Recognition Program** *University Auditorium*

WOMEN'S PROGRAM

Tuesday, June 19

2:30 P.M.—**Talk,** "The Chemist Lends a Hand to Better Living"—MISS BETH PETERSON, *E. I. duPont de Nemours Company, Wilmington, Delaware*
University Auditorium

3:30 P.M.—**Tea**
Student Union

Wednesday, June 20

8:30 A.M.–3:30 P.M.
—**Tour to R. Wallace and Sons Manufacturing Company,** Silversmiths, Wallingford, Conn.
Assemble at *New Haven Hall, North Campus*

9:00 A.M.–3:30 P.M.
—**Tour to Sturbridge Village,** Sturbridge, Massachusetts
Assemble at *New Haven Hall, North Campus*

Thursday, June 21

10:00 A.M.—**Talk,** "New England Landscape"—PROFESSOR HAROLD O. PERKINS, *University of Connecticut*
Student Union 208

2:30 P.M.—**Coffee Hour**
Student Union

CHILDREN'S PROGRAM

CHILDREN 3-5 YEARS

Tuesday, June 19

9:00–11:45 A.M.
—**Supervised Care**
Nursery School

2:00–4:00 P.M.
—**Supervised Care**
Nursery School

Wednesday, June 20

9:00–11:45 A.M.
—**Supervised Care**
Nursery School

2:00–4:00 P.M.
—**Supervised Care**
Nursery School

Thursday, June 21

9:00–11:45 A.M.

—**Supervised Care**
Nursery School

2:00–4:00 P.M.

—**Supervised Care**
Nursery School

CHILDREN 6-8 YEARS

Tuesday, June 19

9:00 A.M.–NOON

—**Campus Hike**
Assemble in *Fairfield Hall Lounge, North Campus*

2:00–2:45 P.M.

—**Recreation**
Athletic Field west of Hawley Armory

3:00–4:00 P.M.

—**Swimming** (Bring towel, suit and health certificate)
Brundage Pool (Men's Physical Education)

7:45–9:00 P.M.

—**Sing and Puppet Show**
Engineering 207

Wednesday, June 20

9:00–10:45 A.M.

—**Handicrafts**
Field House Classroom

11:00 A.M.–12:15 P.M.

—**Tour to Nathan Hale Homestead**
Assemble in *Fairfield Hall Lounge*

12:15–2:00 P.M.

—**Picnic**
Athletic Field

2:00–2:45 P.M.

—**Recreation**
Athletic Field

3:00–4:00 P.M.

—**Swimming**
Brundage Pool

Thursday, June 21

9:30–11:00 A.M.

—**Entertainment**
Field House Classroom

2:00–2:45 P.M.

—**Recreation**
Athletic Field

3:00–4:00 P.M.

—**Swimming**
Brundage Pool

CHILDREN 9-12 YEARS

Tuesday, June 19

9:00 A.M.–NOON

—**Campus Hike**
Assemble in *New Haven Hall Lounge, North Campus*

2:00–2:45 P.M.

—**Recreation**
Athletic Field west of Hawley Armory

3:00–4:00 P.M.

—**Swimming** (Bring towel, suit and health certificate)
Brundage Pool (Men's Physical Education)

7:45–9:00 P.M.

—**Sing and Puppet Show**
Engineering 207

Wednesday, June 20

9:00–11:00 A.M.

—**Tour to Nathan Hale Homestead**
Assemble in *Fairfield Hall Lounge*

11:00 A.M.–NOON

—**Nature Hike**
Assemble in *New Haven Hall Lounge*

12:15–2:00 P.M.

—**Picnic**
Athletic Field

2:00–2:45 P.M.

—**Recreation**
Athletic Field

3:00–4:00 P.M.

—**Swimming**
Brundage Pool

Thursday, June 21 9:30–11:00 A.M.

—**Entertainment**
Field House Classroom

2:00–2:45 P.M.

—**Recreation**
Athletic Field

3:00–4:00 P.M.

—**Swimming**
Brundage Pool

CHILDREN 13 YEARS AND OLDER

Tuesday, June 19 9:00 A.M.–NOON

—**Tour to Nathan Hale Homestead**
Assemble in *Litchfield Hall Lounge, North Campus*

2:00–2:45 P.M.

—**Recreation**
Athletic Field west of Armory

3:00–4:00 P.M.

—**Swimming** (Bring towel, suit and health certificate)
Brundage Pool (Men's Physical Education)

7:45–9:00 P.M.

—**Sing and Puppet Show**
Engineering 207

9:00 P.M.—**Square and Round Dancing**
Hawley Armory (Teen-age Headquarters)

Wednesday, June 20 9:00–11:00 A.M.

—**Recreation**
Athletic Field

11:00 A.M.–NOON

—**Nature Hike**
Assemble in *Litchfield Hall Lounge*

12:15–2:00 P.M.
—**Picnic**
Athletic Field

2:00–2:45 P.M.
—**Recreation**
Athletic Field

3:00–4:00 P.M.
—**Swimming**
Brundage Pool

Thursday, June 21 9:30–11:00 A.M.
—**Entertainment**
Field House Classroom

2:00–2:45 P.M.
—**Recreation**
Athletic Field

3:00–4:00 P.M.
—**Swimming**
Brundage Pool

MANUFACTURING SECTION

Tuesday, June 19

1:30–3:30 P.M.

SECTION A. **Dairy Chemistry**
H. L. TEMPLETON, *Chairman*
Student Union Ballroom

M1. Characterization of phosphorus compounds in alcohol-ether extracts of milk. ALAN KENYON and ROBERT JENNESS, *University of Minnesota.*

M2. Preparation and characterization of two fat-globule membrane fractions from normal milk. C. T. HERALD and J. R. BRUNNER, *Michigan State University.*

M3. Relation between loss in solubility to a fat-protein complex formation. I. I. LITMAN, U. S. ASHWORTH, and H. A. BENDIXEN, *Washington State College.*

M4. Hydrogenation of butter oil. H. D. WEIHE, *Eastern Utilization Research Branch, USDA, Washington, D. C.*

M5. Application of gas-liquid partition chromatography to the study of volatile flavor compounds. W. G. JENNINGS, *University of California.*

M6. Colorimetric determination of fat in milk by the hydroxamic acid reaction. RICHARD BASSETTE AND MARK KEENEY, *University of Maryland.*

M7. Separation of the major components of the milk lipase system by supercentrifugation. W. J. HARPER, I. A. GOULD, and M. BADAMI, *The Ohio State University.*

M8. The water-insoluble acid content of fresh milk. T. R. FREEMAN and W. F. LEWALLEN, *Kentucky Agricultural Experiment Station.*

M9. Effect of time of lactation and of summer and winter feeding on the development of rancidity in milk. E. S. GUTHRIE and B. L. HERRINGTON, *Cornell University.*

1:15–3:15 P.M.

EDUCATION COMMITTEE (JOINT WITH MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)

Selected Topic: Educational Objectives in Dairy Science

E. L. JACK, *Chairman*
Hawley Armory

3:30–4:30 P.M.

Section Business Meeting

W. M. ROBERTS, *Chairman*
Student Union Ballroom

Wednesday, June 20

8:30–10:30 A.M.

SECTION A. JOINT SESSION WITH PRODUCTION AND EXTENSION SECTIONS

Selected Topic: Control of Off-Flavors in Milk

F. J. BABEL, *Chairman*
Student Union Ballroom

Cultural and chemical practices for controlling weeds in pastures. R. A. PETERS, *University of Connecticut.*

Physiological conditions of the cow that influence off flavors. W. E. PETERSEN, *University of Minnesota.*

Hydrolytic rancidity in milk. B. L. HERRINGTON, *Cornell University.*

Equipment for the removal of off-flavors from milk. C. F. WEINREICH, *Cherry-Burrell Corporation, Chicago.*

8:30–10:45 A.M.

SECTION B. **Dairy Chemistry**

H. L. TEMPLETON, *Chairman*
Engineering 207

M10. Changes in the composition of the proteins present in milk during the lactation period. B. L. LARSON, G. D. ROLLERI, and K. A. KENDALL, *University of Illinois.*

M11. The relation of milk serum proteins to the effects of heat treatment on rennet clotting. A. KANNAN and ROBERT JENNESS, *University of Minnesota.*

M12. Physical and chemical properties of some high molecular weight complexes isolated from heated skimmilk. M. J. PALLANSCH, H. S. HALLER, and A. G. MORIN, *Eastern Utilization Research Branch, USDA, Washington, D. C.*

M13. Electrophoretic and sedimentation characteristics of two fat-globule membrane fractions from normal milk. J. R. BRUNNER and C. T. HERALD, *Michigan State University.*

M14. Magnesium in the casein-containing colloid of milk. T. G. ALEXANDER, *Eastern Utilization Research Branch, USDA, Washington, D. C.*

M15. Precipitation of calcium caseinate by heat and subsequent reversal. C. A. ZITTLE, *Eastern Utilization Research Branch, USDA, Philadelphia.*

M16. A study of the dialysis of skimmilk. G. K. MURTHY and R. MCL. WHITNEY, *University of Illinois.*

M17. Cardboard flavor in skimmilk. D. A. FORSS, E. G. PONT, and W. STARK, *Dairy Research Section, C.S.I.R.O., Melbourne, Australia.*

M18. Some observations on gamma radiated skimmilk. E. A. DAY, D. A. FORSS, and STUART PATTON, *The Pennsylvania State University.*

M19. Oxidation of vanillin by unheated milk. S. KURAMOTO, ROBERT JENNESS, and S. T. COULTER, *University of Minnesota.*

11:00 A.M.–NOON

Section Business Meeting
W. M. ROBERTS, *Chairman*
Student Union Ballroom

1:30–4:30 P.M.

SECTION A.
Symposium: Special Dairy Products
H. L. TEMPLETON, *Chairman*
Student Union Ballroom

Flavored milks. A. C. FAY, *H. P. Hood & Sons, Boston.*

Chocolate milk. W. S. MUELLER, *University of Massachusetts.*

Cultured milks. C. B. LANE, *Breakstone Brothers, Inc., Walton, New York.*

Unflavored special milks. ROGER HUNT, *National Dairy Research Laboratories, Oakdale, L. I., New York.*

Sweet high-fat products. G. A. HOURAN, *DeLaval Separator Co., Poughkeepsie, New York.*

Cottage cheese. N. C. ANGEVINE, *Meyer-Blanke and Co., St. Louis, Missouri.*

1:30–4:00 P.M.

SECTION B. **Dairy Bacteriology**
F. J. BABEL, *Chairman*
Engineering 207

M20. The action of penicillin on lactic acid streptococci in milk. C. K. JOHNS, *Canadian Department of Agriculture, Ottawa.*

M21. Host-controlled modification of a bacteriophage without change in poor adsorbability. E. B. COLLINS, *University of California.*

M22. The germicidal property of milk. R. W. HENNINGSON and F. V. KOSIKOWSKI, *Cornell University.*

M23. The influence of heating milk on the subsequent response of starter cultures. V. W. GREENE and J. J. JEZESKI, *University of Minnesota.*

M24. The response of lactic acid bacteria to stimulants present in pancreas tissue. J. K. MCANELLY and M. L. SPECK, *North Carolina State College.*

M25. Factors affecting terramycin activity in milk. K. M. SHAHANI, *The Ohio State University.*

M26. Differences in the phosphorus metabolism of terramycin sensitive and resistant organisms. K. M. SHAHANI and W. J. HARPER, *The Ohio State University.*

M27. Reduction of cottage cheese biacetyl content by organisms associated with cottage cheese spoilage. C. S. WALES and L. G. HARMON, *Michigan State University.*

M28. The effect of various concentrations of sorbic acid at different pH levels on the viability of cottage cheese spoilage organisms. M. D. BONNER and L. G. HARMON, *Michigan State University.*

M29. The influence of environment and processing on spoilage organisms in cottage cheese. L. G. HARMON and C. K. SMITH, *Michigan State University.*

Thursday, June 21

8:30–10:00 A.M.

SECTION A. **Milk**
H. L. TEMPLETON, *Chairman*
Student Union Ballroom

M30. Withdrawn

M31. The effect of variations in fat and temperature upon the surface tension of various milks. P. D. WATSON, *Eastern Utilization Research Branch, USDA, Washington, D. C.*

M32. The use of thermistors for freezing point determinations. W. F. SHIPE, *Cornell University.*

M33. The influence of composition and treatment of milk upon its freezing point. F. PINKERTON and I. I. PETERS, *A. & M. College of Texas.*

M34. The maximum density of milk. C. H. WHITNAH, W. D. RUTZ, and T. M. MEDVED, *Kansas State College.*

M35. Comparison of the Mojonnier, Cenco moisture balance and Karl Fischer titration as methods of determining the total solids in fluid milk. J. B. MICKLE, S. D. MUSGRAVE, R. E. WALTON, F. A. GRAYBILL, M. LOEWENSTEIN, and P. E. JOHNSON, *Oklahoma A. & M. College.*

10:00 A.M.–NOON

GENERAL SESSION DEVOTED TO ASSOCIATION AFFAIRS

I. A. GOULD, *Chairman*
University Auditorium

1:30–4:30 P.M.

SECTION A. **Dairy Products**
F. J. BABEL, *Chairman*
Student Union Ballroom

M36. The protein stability and wettability of milk powder as affected by changes in milk composition. D. CHAKRAVARTI, U. S. ASHWORTH, and H. A. BENDIXEN, *Washington State College.*

M37. A simplified short-time method for making Cheddar cheese from pasteurized milk. H. E. WALTER, A. M. SADLER, J. P. MALKAMES, and C. D. MITCHELL, *Eastern Utilization Research Branch, USDA, Beltsville, Md.*

M38. The influence of milk coagulating enzymes of nonanimal origin upon the flavor and constituents of Cheddar cheese. HAROLD WINDLAN and F. V. KOSIKOWSKI, *Cornell University.*

M39. Formol titration as a measure of changes in protein during cheese ripening. D. G. VAKALERIS and W. V. PRICE, *University of Wisconsin.*

M40. Acidic and neutral carbonyl compounds in various cheese varieties. E. W. BASSETT and W. J. HARPER, *The Ohio State University.*

M41. Studies in the ripening of Limburger cheese. S. L. TUCKEY and M. R. SAHASRABUDHE, *University of Illinois.*

M42. Floating cottage cheese curd made from reconstituted nonfat dry milk solids. A. V. MOORE, *A. & M. College of Texas.*

M43. The use of synthetic β-carotene for coloring butter. R. R. RIEL and C. K. JOHNS, *Canadian Department of Agriculture, Ottawa.*

M44. The influence of various packaging materials on the keeping quality of ice cream and on rates of temperature change during hardening and subsequent handling. V. S. PACKARD, JR., E. L. THOMAS, and W. B. COMBS, *University of Minnesota.*

M45. A process for manufacturing a high nitrogen feed supplement from whey. D. R. ARNOTT, STUART PATTON, and E. M. KESLER, *The Pennsylvania State University.*

M46. Evaluation of the cleanability of CIP automatic valves. D. A. SEIBERLING and W. J. HARPER, *The Ohio State University.*

2:00–4:00 P.M.

JOINT SESSION WITH PRODUCTION AND EXTENSION

Selected Topic: Aspects of Mastitis Control

V. R. SMITH, *Chairman*

Ratcliffe Hicks Livestock Arena

PRODUCTION SECTION

Tuesday, June 19

1:15–5:00 P.M.

SECTION A. **Reproduction and Artificial Insemination**

N. P. RALSTON, *Chairman*

Engineering 207

P1. The influence of oxytocin and epinephrine upon uterine motility and metabolism. M. A. INCHIOSA and N. L. VANDEMARK, *University of Illinois.*

P2. The induction of estrus in cows by electrical stimulation. R. L. HAYS and C. H. CARLEVARO, *University of Illinois.*

P3. Luminal contents of estrous and pseudopregnant rabbit uteri as bacterial culture media. H. W. HAWK, J. SIMON, S. H. MCNUTT, and L. E. CASIDA, *University of Wisconsin.*

P4. Progesterone and B vitamins as factors in maintaining pregnancy in the underfed rat. K. A. KENDALL and R. L. HAYS, *University of Illinois.*

P5. Split-ejaculate fertility comparison between unfrozen and frozen bull semen. J. A. WILLIAMS, *Michigan State University,* and R. W. GREEN, *Michigan Artificial Breeders Cooperative, East Lansing, Michigan.*

P6. The effect of dilution temperatures and cooling rates upon the survival of frozen and unfrozen sperm. E. W. SWANSON and R. H. WHITE, *University of Tennessee.*

P7. The effect of collection interval upon some characteristics of bovine spermatozoa and their survival through freezing. F. I. ELLIOTT, E. J. ELLIOTT, J. I. OHMS, and E. L. WILLETT, *American Foundation for the Study of Genetics, Madison, Wisconsin.*

P8. The daily rate of sperm production in dairy bulls. L. J. BOYD and N. L. VANDEMARK, *University of Illinois.*

P9. Bicarbonate buffer, glycine, and versene in diluters for bull semen. E. L. WILLETT and J. I. OHMS, *American Foundation for the Study of Genetics, Madison, Wisconsin.*

P10. Mineral levels of bovine semen and seminal plasma. R. G. CRAGLE and J. H. MUNTZ, *University of Illinois.*

P11. Motility of sperm in milk diluent with and without seminal plasma. J. L. ALBRIGHT, M. H. EHLERS, and R. E. ERB, *State College of Washington.*

P12. Uptake of phosphorous^{-32} from egg yolk phospholipids by bovine spermatozoa. M. D. CRAWFORD, R. J. FLIPSE, and J. O. ALMQUIST, *Pennsylvania Agricultural Experiment Station.*

P13. The metabolism of bull semen at low temperatures in saline and phosphate-containing diluents. A. W. BLACKSHAW and G. W. SALISBURY, *University of Illinois.*

P14. Pathways of glucose catabolism in bovine spermatozoa. R. J. FLIPSE, *Pennsylvania Agricultural Experiment Station.*

P15. Viability of spermatozoa in bovine genital tract fluids. DURWARD OLDS, *University of Illinois.*

P16. The metabolic activity of semen collected in different seasons of the year. N. T. NAKABAYASHI and G. W. SALISBURY, *University of Illinois.*

1:15–5:00 P.M.

SECTION B. **Milk Secretion**

S. W. MEAD, *Chairman*

Ratcliffe Hicks Livestock Arena

P17. Evaluation of the thyroid activity of dairy animals. G. W. PIPES, *University of Missouri.*

P18. The role of pituitary gland in mammary gland growth. R. P. REECE, *New Jersey Agricultural Experiment Station, New Brunswick.*

P19. Stimulation of udder growth and lactation in infertile dairy heifers. H. L. RUPPERT, JR., H. YAMAMOTO, and C. W. TURNER, *University of Missouri.*

P20. Some effects of feeding diethylstilbestrol to dairy cattle. C. B. Browning, G. B. Marion, F. C. Fountaine, and H. T. Gier, *Kansas State College.*

P21. Effect of feeding stilbestrol to lactating dairy cows. J. F. Sykes, T. R. Wrenn, E. A. Kane, and W. C. Jacobson, *Dairy Husbandry Research Branch, USDA.*

P22. Estrogen content of milk of open and pregnant cows and effect of synthetic estrogen feeding. C. W. Turner, *University of Missouri.*

P23. The mode of formation of milk fat. S. Lakshmanan and J. C. Shaw, *University of Maryland.*

P24. The preparation of carbon^{14} labeled uridine diphosphate glucose for a study of lactose metabolism. S. K. Murthy and R. G. Hansen, *University of Illinois.*

P25. Appearance of the specific blood serum proteins in the lacteal secretions near parturition. B. L. Larson, G. D. Rolleri, and K. A. Kendall, *University of Illinois.*

P26. Preliminary repeatability estimates of per cent total protein and certain other milk constituents of Holstein cows at selected intervals of lactation. S. D. Musgrave, R. E. Walton, F. A. Graybill, R. MacVicar, J. B. Mickle, M. Loewenstein, and P. E. Johnson, *Oklahoma Agricultural Experiment Station.*

P27. Relationship of milk let-down to certain other phenomena of lactation. J. D. Donker, W. J. Miller, H. L. Dalton, and I. Levy, *University of Georgia.*

P28. Effect of vacuum and pulsation rate on rate of milking. W. E. Stewart and L. H. Schultz, *Cornell University.*

P29. Effect of variants in pipeline installations on the acid degree of milk. Louis Jokay and J. M. Jensen, *Michigan State University.*

P30. The resistance of milk samples to the activity of mastitis staphylococci. W. D. Pounden and Norma A. Frank, *Ohio Agricultural Experiment Station, Wooster.*

P31. Comparison of antibacterial activity of mastitis products by an in vitro method. K. E. Price, Z. Zolli, M. J. Gallian, and G. Shea, *Charles Pfizer and Company, Inc., Terre Haute, Indiana.*

1:15–3:15 P.M.

Education Committee (Joint with Manufacturing, Production, and Extension Sections)

Selected Topic: Educational Objectives in Dairy Science

E. L. Jack, *Chairman*

Hawley Armory

Wednesday, June 20

8:00-11:00 A.M.

SECTION A. **Calf Nutrition**
S. W. MEAD, *Chairman*
College of Agriculture Auditorium

P32. The development of rumen microorganisms in inoculated vs. isolated growing calves. M. P. BRYANT and NOLA SMALL, *Dairy Husbandry Research Branch, USDA.*

P33. Absorption of volatile fatty acids from the reticulo-rumen of young dairy calves. W. P. FLATT, R. G. WARNER, and J. K. LOOSLI, *Cornell University.*

P34. Studies using synthetic milks for dairy calves. J. W. THOMAS and M. OKAMOTO, *Dairy Husbandry Research Branch, USDA.*

P35. The value of distillers solubles and vitamin B_{12} in milk replacement formulas with and without alfalfa hay for young dairy calves. G. F. FRIES, G. M. WARD, and C. F. HUFFMAN, *Michigan State University.*

P36. A study of the lipolysis induced in various milks by pregastric esterases obtained from suckling mammals. M. G. FARNHAM and J. H. NELSON, *Dairyland Food Laboratories, Inc., Waukesha, Wisconsin,* and A. R. KEMP, *Dean Milk Company, Rockford, Illinois.*

P37. The influence of chlortetracycline on whole blood, plasma and corpuscle glucose relationships in calves fed high roughage pellets. J. H. VANDERSALL, J. W. HIBBS, and H. R. CONRAD, *Ohio Agricultural Experiment Station, Wooster.*

P38. Effect of chlortetracycline and diethylstilbestrol on growth, carcass, and endocrine glands of dairy calves when fed singly and in combination. BARNEY HARRIS, JR. and L. L. RUSOFF, *Louisiana Agricultural Experiment Station.*

P39. Effects of oral administration of diethylstilbestrol on young dairy calves. H. H. VOELKER and A. E. DRACY, *South Dakota State College.*

P40. A comparison of three levels and two sources of crude fiber in calf starters. R. T. WHITAKER, W. J. MILLER, H. L. DALTON, and J. L. CARMON, *University of Georgia.*

P41. High-moisture vs. wilted grass silage for raising dairy calves. J. A. NEWLANDER and W. H. RIDDELL, *University of Vermont.*

P42. The influence of chlorinated napthalenes on the utilization of vitamin A by calves and sheep. R. G. WARNER, K. L. MCENTEE, WM. HANSEL, and C. H. GRIPPIN, *Cornell University.*

P43. Relation of various nutritional factors to diarrhea in the young calf. F. G. OWEN, N. L. JACOBSON, and R. S. ALLEN, *Iowa State College.*

8:00–11:00 A.M.

SECTION B. **Genetics**

N. P. RALSTON, *Chairman*

Ratcliffe Hicks Livestock Arena

P44. Chest girth–weight relationships for Holstein, Jersey, Guernsey, and Ayrshire males. H. P. DAVIS, *University of Nebraska.*

P45. Factors affecting birth weight of calves in a crossbreeding experiment. T. G. MARTIN, *Purdue University.*

P46. A study of sire influence on rate of maturity in several Holstein-Friesian families. H. L. BARR, T. M. LUDWICK, and FORDYCE ELY, *The Ohio State University* and *Ohio Agricultural Experiment Station* in cooperation with the *Dairy Husbandry Research Branch, USDA.*

P47. The reliability of some intra-herd production information used in selecting sires for use in artificial insemination. H. W. CARTER, R. ALBRECTSEN, C. R. HENDERSON, and J. T. GODFREY, *Cornell University.*

P48. Estimates of genetic progress in the development of the American Red Danish Cattle. N. R. THOMPSON and N. P. RALSTON, *Michigan State University.*

P49. Relations between monthly test day milk production of Holstein-Friesian cows. D. E. MADDEN, L. D. MCGILLIARD, and N. P. RALSTON, *Michigan State University.*

P50. Causes of variation in calving interval of Holstein-Friesian cows. J. C. RENNIE, *Iowa State College.*

P51. Interrelationships between productivity and gestation lengths in dairy cattle. R. C. LABEN, W. C. ROLLINS, and S. W. MEAD, *University of California.*

P52. The influence of season of freshening on production records of Jersey and Holstein cows in Louisiana. J. E. JOHNSTON, CLYDE LEWIS, E. J. STONE, and CECIL BRANTON, *Louisiana Agricultural Experiment Station.*

P53. Mammary gland measurements in calves and their prediction value in herds of varying environment. V. L. BALDWIN, W. E. PETERSEN, and N. D. BAYLEY, *University of Minnesota.*

P54. Withdrawn

P55. The influence of heredity and environment on the fertility of dairy cattle. CECIL BRANTON, W. S. GRIFFITH, H. W. NORTON, and J. G. HALL, *Louisiana Agricultural Experiment Station.*

P56. The amount of pigmented body area in spotted cattle as associated with head pattern and as influenced by sex. J. M. TREECE, L. O. GILMORE, R. LAIRD, and N. S. FECHHEIMER, *Ohio Agricultural Experiment Station, Wooster.*

P57. Progress report—Studies of total milk solids as related to inheritance. H. S. WILLARD, W. R. THOMAS, and D. C. BROWN, *Wyoming Agricultural Experiment Station.*

8:30–10:30 A.M.

JOINT SESSION WITH MANUFACTURING AND EXTENSION SECTIONS

Selected Topic: Control of Off-Flavors in Milk

F. J. BABEL, *Chairman*

Student Union Ballroom

11:00 A.M.–NOON

Section Business Meeting

N. P. RALSTON, *Chairman*

College of Agriculture Auditorium

1:15–3:15 P.M.

SECTION A. **Ketosis and General Physiology**

S. W. MEAD, *Chairman*

College of Agriculture Auditorium

P58. Peripheral leucocytic picture in ketotic cows. G. S. BAJWA, R. A. GESSERT, and A. C. CHUNG, *University of Maryland.*

P59. Adrenal cortical atrophy induced on goats by per os administration of 1, 1-dichloro-2, 2-bis (*p*-chlorophenyl) ethane (DDD or TDE). B. C. HATZIOLOS, *University of Maryland.*

P60. The effect of cobaltous chloride administration on blood sugar and on alpha cells of the pancreatic islets of goats. B. C. HATZIOLOS, *University of Maryland.*

P61. Bromsulphalein elimination rate in dairy cattle with simultaneous determination of liver function and blood and plasma volumes. J. P. MIXNER and W. G. ROBERTSON, *New Jersey Agricultural Experiment Station, Sussex.*

P62. Determination of liver function and plasma and blood volumes in ketotic cows by using bromsulphalein. W. G. ROBERTSON, W. W. BAILEY, H. D. LENNON, JR., and J. P. MIXNER, *New Jersey Agricultural Experiment Station, Sussex.*

P63. Some results of parathyroidectomy of ruminants. V. R. SMITH and G. H. STOTT, *University of Wisconsin.*

P64. A cyto-physiological study of the bovine parathyroid glands. G. H. STOTT and V. R. SMITH, *University of Wisconsin.*

P65. Evaporative cooling in Indian- and European-evolved cattle. R. G. YECK and SAMUEL BRODY, *USDA and University of Missouri.*

1:15–3:15 P.M.

SECTION B. **Pasture and General Nutrition**
N. P. RALSTON, *Chairman*
Ratcliffe Hicks Livestock Arena

P66. Estimation of dry matter digestibility by employing randomly procured samples of feces. W. A. HARDISON, W. N. LINKOUS, and C. Y. WARD, *Virginia Agricultural Experiment Station.*

P67. The utilization of a mixture of alfalfa *(Medicago falcata)* and smooth bromegrass *(Bromus inermis)* under rotational and strip grazing systems of pasture management. A. L. BRUNDAGE and W. J. SWEETMAN, *Alaska Agricultural Experiment Station, Palmer.*

P68. Progress report on soilage feeding of dairy cattle. B. W. HENDERSON, J. W. COBBLE, and H. J. COOK, *University of Rhode Island.*

P69. A comparison of summer annuals for milking cows. B. F. HOLLEN, W. W. WOODHOUSE, and R. D. MOCHRIE, *USDA* and *North Carolina State College.*

P70. The effect of fine grinding of hay on the digestibility of its nutrients and rate of passage through the digestive tract. C. B. RODRIGUE and N. N. ALLEN, *University of Wisconsin.*

P71. Effects of fiber content of the diet on milk production and hot weather discomfort in producing dairy cows. R. E. LEIGHTON and I. W. RUPEL, *Texas Agricultural Experiment Station.*

P72. Hay consumption of individual cows on limited grain feeding. J. E. LEGATES, W. R. MURLEY, and R. K. WAUGH, *North Carolina State College.*

P73. Variation in efficiency of feed utilization for maintenance of the dairy cow. J. W. THOMAS and L. A. MOORE, *Dairy Husbandry Research Branch, USDA.*

P74. Bone changes in molybdenum toxicity. H. F. ROBERTS, L. R. ARRINGTON, C. F. SIMPSON, and G. K. DAVIS, *Florida Agricultural Experiment Station.*

P75. Dried extracted coffee meal as a feed for dairy cattle. R. E. MATHER and W. P. APGAR, JR., *New Jersey Agricultural Experiment Station, Sussex.*

3:30–4:30 P.M.

JOINT SESSION WITH EXTENSION SECTION

Joint Committee Reports

G. M. WERNER, *Chairman*
Ratcliffe Hicks Livestock Arena

Breeds Relations. G. H. BECK, *Kansas State College*

Dairy Cattle Breeding. E. C. SCHEIDENHELM, *University of Kentucky*

Dairy Cattle Type. W. J. TYLER, *University of Wisconsin*

Dairy Cattle Health. F. J. ARNOLD, *Iowa State College*

Thursday, June 21

8:00–10:00 A.M.

SECTION A. **Nutrition and Silage**

N. P. RALSTON, *Chairman*
College of Agriculture Auditorium

P76. Grass-legume silage as the sole roughage for lactating cows. J. W. COBBLE and GLENN WILDES, *University of Rhode Island.*

P77. Effects of sodium metabisulfite and iodized sodium chloride on preservation and milk production value of alfalfa silage with levels of protein supplementation. H. H. VOELKER and E. R. BECKER, *South Dakota State College.*

P78. Effect of various preservatives on seepage from high moisture silage. P. M. REAVES and R. E. BRUBAKER, *Virginia Polytechnic Institute.*

P79. Digestibility of grass and legume silages preserved with sodium metabisulfite and dried molasses beet pulp. I. R. JONES, J. V. BATEMAN, and J. R. SCHUBERT, *Oregon State College.*

P80. A comparison of chopped and unchopped silage stored in bunker silos. C. H. GORDON, H. G. WISEMAN, and C. G. MELIN, *Dairy Husbandry Research Branch, USDA* and R. J. MCCALMONT and L. E. CAMPBELL, *Agricultural Engineering Research Branch, USDA.*

P81. Effect of grain on the digestibility of grass silage. E. A. KANE *and* W. C. JACOBSON, *Dairy Husbandry Research Branch, USDA.*

P82. Ear corn silage as grain for dairy cattle. A. D. PRATT and C. F. ROGERS, *Ohio Agricultural Experiment Station, Wooster.*

P83. Development of organic acids in silage. H. M. IRVIN, C. W. LANGSTON, and C. H. GORDON, *Dairy Husbandry Research Branch, USDA.*

P84. Chromatographic separation of silage acids. H. G. WISEMAN and H. M. IRVIN, *Dairy Husbandry Research Branch, USDA.*

10:00 A.M.–NOON

GENERAL SESSION DEVOTED TO ASSOCIATION AFFAIRS

I. A. GOULD, *Chairman*
University Auditorium

1:15–4:30 P.M.

SECTION A. **Ruminant Nutrition**
S. W. MEAD, *Chairman*
College of Agriculture Auditorium

P85. In vitro studies on methanogenic rumen bacteria. W. O. NELSON, R. A. OPPERMANN, and R. E. BROWN, *University of Illinois.*

P86. Mechanism of methane formation by bovine rumen bacteria and the effect of hydrogen acceptors and donors on methane production. J. J. MCNEILL and R. N. DOETSCH, *University of Maryland.*

P87. The effect of d-limonene on in vitro dissimilation of carbohydrates and methane formation by rumen bacteria. R. E. BROWN, ANATOLE CRANE, and W. O. NELSON, *University of Illinois.*

P88. Nutritional requirements of rumen bacteria. J. J. GILROY and R. N. DOETSCH, *University of Maryland.*

P89. A time study of the relative concentrations of fatty acids occurring in the rumen of cattle on different rations. T. N. WEGNER, G. M. WARD, and L. E. WASHBURN, *Colorado A & M College.*

P90. Blood and rumen substances of cows on different feeding regimes prepartum and postpartum. T. R. LEWIS and D. R. JACOBSON, *University of Maryland.*

P91. Absorption and dissimilation of lactates added to the bovine rumen and the resulting effects on blood glucose. F. G. HUETER, *University of Maryland.*

P92. Changes in rumen and urine nitrogen and volatile fatty acids in relation to time after feeding alfalfa or mixed hay. H. R. CONRAD and J. W. HIBBS, *Ohio Agricultural Experiment Station, Wooster.*

P93. Effect of detergent on incidence and severity of bloat and on characteristics of rumen fluid of cattle. T. J. BLAKE, R. S. ALLEN, and N. L. JACOBSON, *Iowa State College.*

P94. Factors affecting the dry matter digestion of various roughages in the artificial rumen. B. R. BAUMGARDT and D. L. HILL, *Purdue University.*

P95. An inexpensive urine collecting apparatus for dairy cows. P. A. PUTNAM, R. G. WARNER, and J. K. LOOSLI, *Cornell University.*

2:00–4:00 P.M.

JOINT SESSION WITH MANUFACTURING AND EXTENSION SECTIONS

Selected Topic: Aspects of Mastitis Control

V. R. SMITH, *Chairman*

Ratcliffe Hicks Livestock Arena

EXTENSION SECTION

Tuesday, June 19

1:30–3:30 P.M.

Opening Business Session

G. M. WERNER, *Chairman*

College of Agriculture Auditorium

Dairy Records Committee Report

L. A. JOHNSON, *Chairman*

1:15–3:15 P.M.

EDUCATION COMMITTEE (JOINT WITH MANUFACTURING, PRODUCTION, AND EXTENSION SECTIONS)

Selected Topic: Educational Objectives in Dairy Science

E. L. JACK, *Chairman*

Hawley Armory

3:40–5:00 P.M.

Presentation and Discussion of Exhibits

W. R. HESSELTINE, *Chairman*

College of Agriculture Auditorium

Wednesday, June 20

8:30–10:45 A.M.

4-H Program

J. D. GEORGE, *Chairman*

Ratcliffe Hicks 10 and 11

E1. 4-H dairy calf placement programs. R. A. PORTERFIELD, *The Ohio State University.*

E2. A look at our junior dairy show program—A Panel

H. A. WILLMAN, *Cornell University, Moderator*

H. L. MANN, *Pennsylvania State University*

C. W. NIBLER, *University of Nebraska*

W. N. PATTERSON, *Virginia Polytechnic Institute*
FRED GAUNT, *Brown Swiss Cattle Breeders' Association, Milltown, New Jersey*

8:30–10:30 A.M.

JOINT SESSION WITH MANUFACTURING AND PRODUCTION SECTIONS

Selected Topic: Control of Off-Flavors in Milk
F. J. BABEL, *Chairman*
Student Union Ballroom

11:00 A.M.–NOON

Section Business Meeting and Committee Reports
G. M. WERNER, *Chairman*
Ratcliffe Hicks 10 and 11

1:15–3:15 P.M.

Parasite Control in Dairy Cattle and Special Quality Milk Program to Control Flavors
LEO FRYMAN, *Chairman*
Ratcliffe Hicks 10 and 11

E3. Worm parasite control in dairy management. A. C. TODD, *University of Wisconsin.*

E4. Putting flavor into the quality program. ALEC BRADFIELD, *University of Vermont.*

E5. Getting dairymen milk-flavor conscious. W. A. DODGE, *University of Vermont.*

3:30–4:30 P.M.

JOINT SESSION WITH PRODUCTION SECTION

Joint Committee Reports
G. M. WERNER, *Chairman*
Ratcliffe Hicks Livestock Arena

Breeds Relations. G. H. BECK, *Kansas State College.*
Dairy Cattle Breeding, E. C. SCHEIDENHELM, *University of Kentucky.*
Dairy Cattle Type. W. J. TYLER, *University of Wisconsin.*
Dairy Cattle Health. F. J. ARNOLD, *Iowa State College.*

4:30–5:30 P.M.

MEETING OF SUPERINTENDENTS OF OFFICIAL TESTING
L. A. JOHNSON, *Chairman*
Ratcliffe Hicks Livestock Arena

Thursday, June 21

8:30–9:50 A.M.

Sire Evaluation and Management Factors Influencing Yields

J. D. BURKE, *Chairman*
Ratcliffe Hicks Livestock Arena

E6. Some environmental adjustments which aid in sire evaluation. H. W. CARTER, *Cornell University.*

E7. Demonstration of some management influences on lactation yields (D.H.I.A. 718's). MARY M. GOODWIN and R. E. ERB, *State College of Washington.*

10:00 A.M.–NOON

GENERAL SESSION DEVOTED TO ASSOCIATION AFFAIRS

I. A. GOULD, *Chairman*
University Auditorium

1:15–1:45 P.M.

Management

G. M. WERNER, *Chairman*
Ratcliffe Hicks Livestock Arena

E8. Costs and returns from pasture on Florida dairy farms. C. W. REAVES, *University of Florida.*

2:00–4:00 P.M.

JOINT SESSION WITH MANUFACTURING AND PRODUCTION SECTIONS

Selected Topic: Aspects of Mastitis Control

V. R. SMITH, *Chairman*
Ratcliffe Hicks Livestock Arena

E9. The place of herd management in mastitis control. J. F. CONE, *Pennsylvania State University.*

E10. Diagnosis and treatment of mastitis. W. N. PLASTRIDGE and H. H. HALE, *University of Connecticut.*

E11. A state mastitis control program. H. C. HODGES, *Cornell University.*

SYNTHETIC MILK FOR THE STUDY OF CHEESE MAKING. I. PREPARATION OF A CASEIN SOL IN SEMBLANCE OF SKIMMILK[1]

P. E. GRINDROD,[2] W. V. PRICE, AND H. H. SOMMER[3]
Department of Dairy and Food Industries, University of Wisconsin, Madison

For many centuries man's knowledge of cheese making accumulated by trial and error. Application of the experimental method in recent years has hastened progress despite limitations imposed by variability of milk and biological processes.

It seemed possible that the use of synthetic milk might simplify experimental procedures of studying cheese making. Such "milk" would be synthesized from components of known identity and purity. Bacteriological growth factors would be incorporated as required. Effects of concentrations and functions of major and minor constituents of milk might thus be studied.

Normal skimmilk is an unstable biological fluid. Some of its constituents are in solution, others in colloidal suspension of different degrees of dispersion. The preparation of a synthetic skimmilk must observe certain conditions: The order of salt addition must permit the precipitation of the ion components of the least soluble salts, for example Ca^{++} and $PO_4\equiv$, in stable colloidal form in the presence of soluble caseinates, and must not cause irreversible changes in the casein; the order of salt incorporation must accomplish control of pH to minimize casein hydrolysis while increasing casein re-solution; temperatures used must increase solubility of the less soluble milk salts, including proteinates, without decreasing rates of chemical reaction to an impractical point, and must minimize casein hydrolysis while accelerating the re-solution of the casein; and mass transfer must be accelerated sufficiently by agitation.

Casein sols have been made experimentally to simulate some of the properties of skimmilk. Clark *(2)* prepared a casein sol that exhibited many properties of skimmilk but not its salt composition. Svedberg *et al. (12)*, Snyder *(9)*, Albrecht *(1)*, and Verma *(15)* prepared casein sols of various compositions, none of which approached the composition of skimmilk.

The study reported here was made to develop a casein sol with certain properties similar to those of milk.

MATERIALS AND METHODS

Casein. Casein was isolated from 50-gal. lots of skimmilk obtained from the mixed milk of several herds of normal, healthy cows; a modification of the Van Slyke and Baker *(14)* method of preparing isoelectric casein was used. The washed casein was not extracted with ether and alcohol and air dried; instead, the casein was repeatedly washed with distilled water to effect a calculated dilu-

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[1] This project was supported in part by the Research Committee of the Graduate School with funds from the Wisconsin Alumni Research Foundation.
[2] Present address: C. J. Berst and Company, Portage, Wis.
[3] Deceased.

tion of water solubles in excess of 1:3,000. The isoelectric casein was dried to about 75% moisture by pressing and then frozen rapidly at –25° C. in closed glass bottles containing approximately 350 g.

The freezing and storing of the isoelectric casein in this manner had no obvious effect on the physical appearance or structure of the casein after defrosting. Comparisons of the frozen versus fresh or nonfrozen casein showed that the freezing treatment had no detectable influence on the reaction to rennet of casein sols made from them. This method of preservation was chosen to avoid the hazards of possible chemical changes caused by drying, chemical dehydration, or bacterial or enzymatic action at storage temperatures slightly above 0° C.

Electrophoretic patterns of this casein were made by extracting it with alcohol and ether, filtering, and air drying at room temperature to 5% moisture. The patterns were made from 1% solutions of casein in Tiselius-Kabat buffer *(13)* at pH 6.85 and in a phosphate buffer made from 0.025 *M* NaH_2PO_4 and sufficient Na_2HPO_4 to adjust the pH to 7.0.

The coagulating properties of milk and casein sol were measured by time of coagulation with rennet *(11)*, by the rennet, curd-tension test *(6)*, and by the pepsin-hydrochloric acid, curd-tension test *(3)*.

Butter oil. Butter oil, which was emulsified into the casein sols, was prepared from freshly churned, unsalted butter *(8)*. Soybean phospholipids for emulsifying the butter oil were extracted from crude commercial soybean by repeatedly dissolving in ether and precipitating in acetone until a colorless product was obtained.

Whey protein. Whey protein was isolated from skimmilk from the same source as that used for making the casein. The casein was first precipitated from the skimmilk at 10° to 15° C. by adding 2 to 3 *N* HCl until a pH of 4.6 was obtained. The resulting whey was filtered, concentrated by freezing out ice, dialyzed until free of chlorides, further concentrated by pervaporation, dialyzed against distilled water for 3 days and, finally, lyophilized and stored in closed glass bottles at 2° C.

The pH measurements of all solutions were made by glass electrode and model G or H2 Beckman pH meters.

Moisture determinations on casein were made by drying 2-g. samples in 50-ml. Pyrex beakers at 110° C. for 16 ± 2 hours in a forced draft oven.

Compounding the casein sol. A method of redispersing casein in cold $Ca(OH)_2$ solution was described by Clark *(2)*. He showed the necessity of following a definite order of combining salt components. This sol contained the sulfate ion, which is not normally found in milk. Clark's method, however, suggested the following procedure, which, after many trials and modifications, was finally adopted for preparing a casein sol with salt composition in semblance of skimmilk.

Composition. The composition chosen for the sol conformed to that of skimmilk derived from whole milk containing 3.9% fat. Such milk was described by Sommer *(10)*. The calculated composition of the desired sol was: CaO – 0.177%, Na_2O – 0.02728%, K_2O – 0.182%, and MgO – 0.0177%; P_2O_5 – 0.182%, citric acid – 0.208%, and Cl – 0.104%; casein – 2.6%, and lactose – 5.3%. The P_2O_5

TABLE 1

The composition of the four solutions of reagents used in the preparation of the casein sol

Solution No.	Reagent	Concentration, molarity at 2° C.
1	MgO	0.0878
	Citric acid · HOH	0.217
	H_3PO_4	0.513
	KOH	0.573
2	$CaCl_2 \cdot 2HOH$	0.586
3	KOH	0.400
	NaOH	0.940
4	$Ca(OH)_2$	0.0247

concentration indicated represents the difference between the total P_2O_5 content of average milk and the sum of the P_2O_5 in isoelectric casein and in the phospholipids of normal skimmilk.

Salts. Four solutions were made at 2° C. to accomplish the preparation of the sol; their compositions are shown in Table 1.

Blending. The casein sol was prepared in units of 1,000 g. All measurements of its components were made at 2° C. The necessary amount of freshly defrosted casein was weighed into a 2-l. beaker, and to it was added 684 ml. of solution 4. This mixture was agitated with a 4-cm. propeller turning at 1,520 r.p.m. while 25 ml. of solution 3 was added. Agitation was continued for 20 to 25 minutes until complete dispersion of the casein was attained, as evidenced by visual inspection. Twenty-five ml. of solution 2 was added and followed by an additional 5 minutes of stirring. Fifty ml. of solution 1 was next added slowly, and in order to disperse it more thoroughly it was directed from the pipette against the rotating propeller shaft where it entered the mixture. After 5 more minutes of stirring, the required quantity of lactose was added, and the total weight of the sol was adjusted to 1,000 g. with distilled water. Agitation was continued until visual observation showed that the lactose was dissolved. The sol was held at 2° C. for 16 hours prior to its use.

RESULTS

Appearance of the casein sol. The casein sol made in this manner resembled watered skimmilk in appearance. Its opaqueness increased at temperatures of 36° to 40° C. After pasteurization, it was practically as opaque as natural skimmilk. The components of the casein sol did not settle out of suspension even after standing 1 week at 2° C.

Electrophoretic patterns of the casein of the sol. Changes in the casein which might be caused by isolation and redispersion would be undesirable for the ultimate purposes of this study. Electrophoretic patterns were made of the isoelectric casein before dispersing it in the casein sol and again after reprecipitating it from the sol. In the same manner patterns were made of the isoelectric casein which had been dispersed in N/10 NaOH at room temperature and then reprecipitated.

Reprecipitating casein from the casein sol. The casein was reprecipitated from the casein sol in a manner similar to that used in isolating the original casein. After refrigerating the freshly prepared sol at 2° C. overnight, 3 l. were slowly acidified to pH 5.2 and held at 15° C. for 3 hours. The mixture was further acidified to pH 4.6 to 4.7, held 4 hours, and then the supernatant was decanted and the casein was washed by the process used in isolating the casein from skimmilk.

Reprecipitating casein from N/10 NaOH suspension. The suspension of casein, which had been made by dissolving 2.5% of the casein in N/10 NaOH at 25° C., was reprecipitated by N/10 HCl added with constant stirring until the pH reached 4.6 to 4.7. The precipitated casein was washed by the process used in isolating the casein from skimmilk.

Casein and casein reprecipitated from the casein sol and N/10 NaOH were extracted with alcohol and ether and then suspended in phosphate buffers at pH 6.85 and 7.00 to determine electrophoretic mobilities.

The electrophoretic mobilities are recorded in Table 2, and the electrophoretic patterns are reproduced in Figures 1 and 2 from tracings of the original negatives.

TABLE 2

The electrophoretic mobility of various casein samples

Source of casein sample	Time in minutes	Buffer conductivity $\times 10^3$ (ohm-1 $\times$ om-1)	Electrophoretic mobility $\times 10^{-5}$	Asymmetry, descending alpha peak
		pH 6.85 phosphate buffer		
Skimmilk	65	3.005	−8.39	no
	125	3.005	−8.40	yes
Casein sol	65	3.005	−8.25	no
	125	3.005	−8.22	no
Suspension in N/10 NaOH	65	2.96	−7.65	no
	125	2.96	−7.55	no
		pH 7.00 phosphate buffer		
Skimmilk	65	2.57	−7.27	yes
	120	2.57	−7.52	yes
Casein sol	65	2.57	−7.41	no
	120	2.57	−7.42	no

The data of Table 2 show that in buffers at pH 6.85 and 7.00 the electrophoretic mobility of casein recovered from a casein sol was practically identical to that of the original casein from skimmilk. This close agreement suggests that the method of preparing the casein sols was not detrimental to the casein. The larger difference between the original casein and that which had been reprecipitated from N/10 NaOH suggests, but does not prove, that such treatment altered the casein.

Figures 1 and 2 show that the original casein from skimmilk had an asymmetric alpha peak in both buffers, an indication of the presence of a foreign substance, probably a protein-fat complex.

The electrophoretic patterns of casein reprecipitated from a casein sol and from N/10 NaOH were symmetrical. This fact is attributed to the re-solution

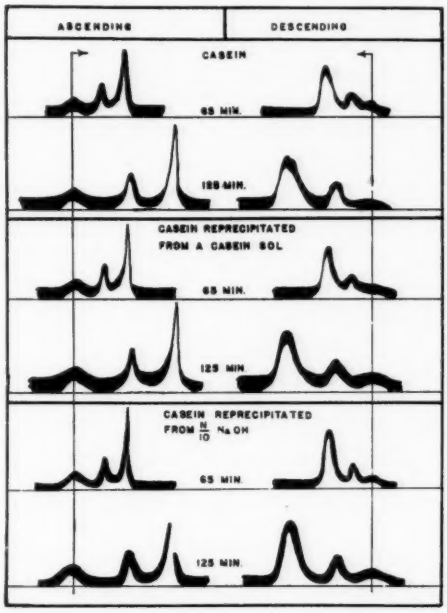

FIG. 1. Electrophoretic patterns of various casein samples in pH 6.85 phosphate buffer.

and reprecipitation of the casein. It is believed that this reprecipitation eliminated the alleged casein-fat complex substance. This resulted in a homogeneous alpha casein fraction.

Curd-making properties of the casein sol. The properties of the casein sol were compared to those of skimmilk by measuring pH, rennet coagulation time,

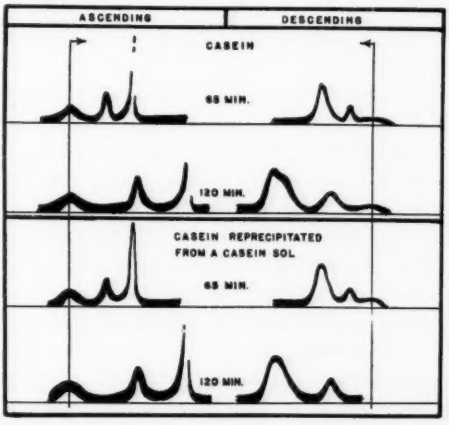

FIG. 2. Electrophoretic patterns of various casein samples in pH 7.00 phosphate buffer.

and rennet curd tension. As a measure of reproducibility, two different sets of stock reagents were used.

Eight sols were prepared independently from one lot of original casein containing 73.71% moisture. The solutions of each of the two sets of stock reagents were used to prepare four replicate casein sols. Each set of four replicates was made on different days. The raw skimmilk for comparative purposes contained approximately 2.3% casein and 8.5% solids-not-fat and was obtained fresh each day from the mixed milk of 40 to 50 herds. Table 3 shows the results of these comparisons.

TABLE 3

The pH, curd-making properties, and reproducibility of separately prepared casein sols

Replicate[a]	Acidity		Rennet coagulation time		Rennet curd tension	
	Casein sol	Skim-milk[b]	Casein sol	Skim-milk[b]	Casein sol	Skim-milk[b]
(No.)	*(pH)*	*(pH)*	*(rev.)*	*(rev.)*	*(g.)*	*(g.)*
A 1	6.68	6.68	198	290	25	8
A 2	6.68	6.68	197	292	30	7
A 3	6.68	6.68	197	290	30	7
A 4	6.68	6.68	197	290	28	6
B 1	6.70	6.69	234	312	20	12
B 2	6.70	6.69	233	310	20	14
B 3	6.70	6.69	233	308	20	13
B 4	6.70	6.69	233	308	22	13

[a] Replicates A1 to A4 were prepared with one set of stock reagents, B1 to B4 with another set of stock reagents. The same casein was used in all casein sols.

[b] One lot of skimmilk was used in the four replicate tests, A1 to A4; another lot of skimmilk provided the four replicates, B1 to B4.

As shown in Table 3, the replicate samples of casein sols exhibited practically identical values of pH, rennet coagulating times, and curd tension measurements. The replicate values were as uniform as those made on identical samples of skimmilk. The casein sols coagulated more quickly and gave higher curd tension values than the natural skimmilk. It should be recalled in this connection that the casein content of the sol was 2.6% so that the differences in curd tension were not unexpected.

Accumulative effects of additives on curd tension. Three normal components of milk were next added to the casein sol. One portion of the casein sol served as a control. To a second portion was added 5.3% of lactose. To a third portion was added 5.3% lactose and 0.7% whey protein. To a fourth portion was added 5.3% lactose, 0.7% whey protein, and 3.5% butter oil.

The whey protein was dispersed in the sol with a Waring blendor. The butter oil was dispersed by the Waring blendor and a laboratory hand homogenizer. The dispersion was checked microscopically until it approximated that of whole milk. The butter oil in the emulsion was stabilized by the addition of 1% of its weight of soy bean phospholipids.

Measurements of curd tension of these four portions of casein sol with their respective additives were made in triplicate by the pepsin-HCl curd tension method.

The curd tension measurements of the casein sol and the sol plus lactose ranged from 42 to 46 and averaged 44 and 43, respectively. The portion containing added lactose and whey protein had a curd tension of 37 g. in all replicates. That portion containing lactose, whey protein and fat, 27 g. of curd tension, and again all replicates were identical.

The results of these trials indicated that the reactions of the casein sol were comparable to those which have been observed with normal milk. Lactose had no effect on curd tension. This observation agrees with the work of Weisberg *et al.(17)*. Whey protein produced a weaker curd, and in this connection it is interesting to note that cheese makers are wary of milk containing large amounts of albumin. The decrease in curd tension by the fat is an effect of common knowledge to all who have standardized the fat content of milk for cheese making.

The experiment does not show whether the order of adding these components affected the accumulative results on curd tension. Interaction of the components might have occurred, and the degree of mechanical dispersion of the butter oil was certainly an important factor. The combination of effects actually produced curd tension approaching the soft-curd classification of Hill *(5)*.

DISCUSSION

The mechanism of redispersion of casein to make a casein sol with properties simulating those of skimmilk seems to depend on a favorable equilibrium for salt solubility and on the decreased rate of casein hydrolysis. When $Ca(OH)_2$, NaOH, and KOH are first added to casein at 2° C., the casein disperses rapidly. Despite the high pH of this mixture, the low temperature minimizes the hydrolysis of casein sufficiently to prevent changes in the electrophoretic pattern and the rennet response of casein.

When the balance of the salt solutions are added to the mixture of casein and calcium, sodium and potassium hydroxides, the highly dispersed casein and salts are condensed or aggregated by the lowered pH. The solubility of casein is reduced by the lowered pH, and the addition of phosphates and citrates results in the formation of the more insoluble milk salts, which condense or aggregate in the presence of the protective colloids, the soluble caseinates. Zoller *(19)* stated that the soluble caseinates are as effective as gelatin and 20 times as effective as gum arabic in preventing the precipitation and flocculation of colloidal gold.

This method of casein sol preparation, involving a definite order of milk salt incorporation at low temperature, was the only method found that produced, without casein hydrolysis, a colloidal dispersion that would respond normally to the action of rennet.

Methods of preparation at room temperature either failed to achieve colloidal dispersion or hydrolyzed the casein. Reversing or altering the order of milk salt incorporation also resulted in failure to achieve colloidal dispersion.

The close agreement between the electrophoretic mobilities of isoelectric casein and the casein reprecipitated from the casein sol suggests that the method

of preparing the casein sols was not detrimental to the protein. Mellander *(7)* determined the electrophoretic mobility of casein fractions in phosphate buffer; interpolation of his values estimates the electrophoretic mobility of the alpha fraction of casein to be -8.71×10^{-5}.

Measurements of electrophoretic mobilities of isoelectric casein and casein reprecipitated from the casein sol in this study were -8.40×10^{-5} and -8.22×10^{-5}, respectively. The agreement between these results and those of Mellander would seem to be reasonably close, considering differences in methods of preparation and in sources of casein.

The asymmetry of the electrophoretic pattern of the isoelectric casein and the symmetry of the descending alpha peak of casein reprecipitated from the casein sol and from the N/10 NaOH indicate interesting differences in these proteins. The asymmetry of the alpha peak of the isoelectric casein is in harmony with the observations of Zehren *(18)*, who studied the electrophoretic patterns of casein prepared by the methods of Hammarsten *(4)*, Van Slyke and Baker *(14)*, and Warner *(16)*.

Zehren found that the asymmetry of the alpha peak of casein could be eliminated by repeated extraction with alcohol and ether.

The absence of asymmetry in the electrophoretic patterns of the alpha casein fractions of the casein, which had been dispersed and then reprecipitated from the casein sol, indicates a method of refinement that might be particularly attractive in basic research in this field of interest.

The data submitted in this study indicate that casein from a constant source can be combined with easily obtainable, purified lactose and salts of milk to make a casein sol of uniform composition and curd-making properties. The reproducibility of this casein sol in any laboratory is predicated upon the assumption that the casein of milk, regardless of its source, is a constant entity. The experiments of this study have not been concerned with broad aspects of of this assumption.

The properties of the casein sol under consideration suggest possibilities of several uses, some of which should be indicated here: This sol might be used for the standardization of rennet extract and for the study of enzymes of comparable characteristics derived from animals, plants, or microorganisms. The sol provides a base to which growth factors might be added to study biological requirements for milk fermentations. With added growth factors to support the activity of organisms involved, this sol might be used as a constant medium for propagating starters. Finally, the casein sol offers an interesting medium to study curd-making processes and the effects of certain milk constituents upon them. Some of these possibilities have been explored and will be described later in another report.

SUMMARY

A method of preparing a casein sol similar in salt composition to skimmilk is described. The success of the method depends upon a definite order of combining salt components with purified casein at a low temperature.

Electrophoretic patterns of casein recovered from the sol show that the method of preparing the sol does not have a detrimental effect upon the casein. The casein sol reacts with rennet like skimmilk in that it shows a similar rate of coagulation and curd tension development. The casein sol resembles milk in that its curd tension is not affected by adding limited amounts of lactose but is decreased by adding whey protein and emulsified butterfat.

The properties of this casein sol suggest that it should be a useful medium for studies of milk-coagulating enzymes, milk fermentations, and curd-making processes.

ACKNOWLEDGMENT

We are indebted to V. L. Zehren, who made the electrophoretic patterns reported here.

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EFFECTS OF LIPOLYTIC ACTIVITY AND OF MERCURIC CHLORIDE ON THE BABCOCK TEST FOR FAT IN COMPOSITE MILK SAMPLES[1]

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Numerous references in the literature show that the Babcock butterfat test of milk composited over a period of days and preserved with mercuric chloride commonly yields results which are lower than the average of the tests of the fresh aliquot portions. The degree of the fat reduction varies with temperature during holding, length of the holding period, amount of mercuric chloride used for preserving, and other factors *(3, 4, 10, 11, 12)*.

Davies *(2)* reported that 10 p.p.m. of copper in the form of copper lactate would completely inactivate lipolytic activity in freshly churned salted butter. The copper lactate was added with the salt. He stated that the depression of lipolytic activity by various metals varied directly with their catalytic power in inducing fat oxidation. He also reported that 60 to 70% of the free fatty acids liberated by lipolytic hydrolysis of butterfat is oleic acid and less than 5% is volatile fatty acids. Krukovsky and Sharp *(6)* and Hlynka and Hood *(5)* showed that dissolved copper does not inactivate lipase in the absence of oxygen. Activity of lipase, according to Roahen and Sommer *(8)*, is greatest at 100° F., slightly less at 80° F., and decidedly less at 38° to 40° F. The end products of the hydrolysis of butterfat include water-soluble fatty acids and glycerides made more soluble by the loss of a fatty acid. The separation of the fatty substances is made more difficult because of this increased solubility. Lipolytic hydrolysis occurs naturally in milk to a varying degree.

This study of the possible effect of lipase activity and of mercuric chloride on the Babcock test was undertaken to determine why the test of stored composite samples is lower than the average test of the fresh aliquots.

EXPERIMENTAL PROCEDURE

Reductions in the butterfat tests and corresponding increases in free fatty acids were determined for single and composite samples with split samples of raw Grade A milk from the College dairy.

In the first experiment (Figure 1) daily samples were obtained at random from 10-gal. cans of milk. Each sample was divided into four portions of 440 ml. each, and the portions were treated as follows: *(a)* no additions; *(b)* addition of two No. 2 corrosive sublimate ($HgCl_2$) preservative tablets (0.45 g. each); *(c)* addition of two No. 2 corrosive sublimate tablets plus 20 p.p.m. copper in the form of copper sulfate; and *(d)* addition of two No. 2 corrosive sublimate tablets after heating the milk to 160° F. for 30 to 60 seconds and cooling to 60°

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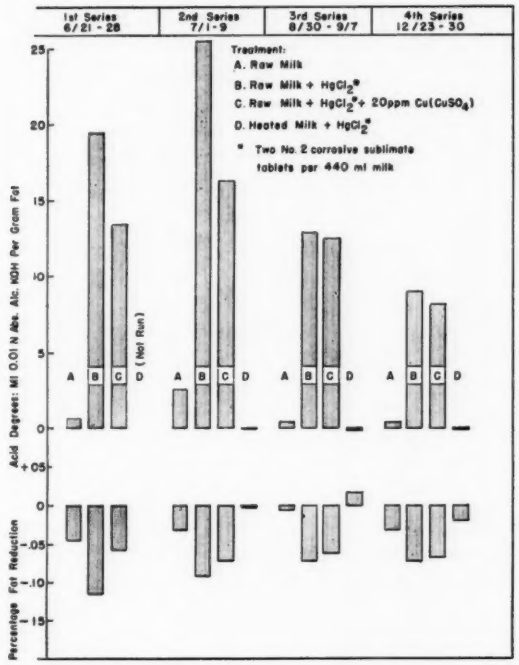
1st Series 6/21 - 28
2nd Series 7/1 - 9
3rd Series 8/30 - 9/7
4th Series 12/23 - 30
Treatment:
A. Raw Milk
B. Raw Milk + $HgCl_2$*
C. Raw Milk + $HgCl_2$*+ 20ppm Cu($CuSO_4$)
D. Heated Milk + $HgCl_2$*
* Two No. 2 corrosive sublimate tablets per 440 ml milk
Acid Degrees: Ml 0.01 N Alc. KOH Per Gram Fat
Percentage Fat Reduction
D (Not Run)

FIG. 1. Increase in free fatty acids compared with Babcock fat test reduction during 7-8 day compositing period (5 aliquot portions).

to 70° F. After all additives had been completely dissolved, each portion was tested immediately in triplicate for butterfat by the Babcock method and in duplicate for free fatty acids by a method described later in this section. In the first experiment (Figure 1) samples were obtained at random from 10-gal. cans of milk on each of five successive days. Composites were prepared from each portion. During the compositing period the composites were held at 35° to 38° F. On the seventh or eighth day the composites were tested in triplicate for butterfat and in duplicate for free fatty acid content. Four composite sets were prepared in this manner.

In a second experiment (Figure 2) a daily fresh sample was obtained at random on five different days from 10-gal. cans of milk and split into two portions of 440 ml. each: *(a)* with the addition of two No. 2 corrosive sublimate tablets and *(b)* with the addition of two No. 2 corrosive sublimate tablets after heating the milk to 160° F. for 60 seconds and cooling to 60°-65° F. Each portion was tested when fresh and at the end of the compositing period in triplicate for butterfat and in duplicate for free fatty acid content. Composites were made from the five split samples during a 15-day period. The composites were held at 35° to 38° F. On the 15th day, the composites were tested for butterfat and free fatty acid content.

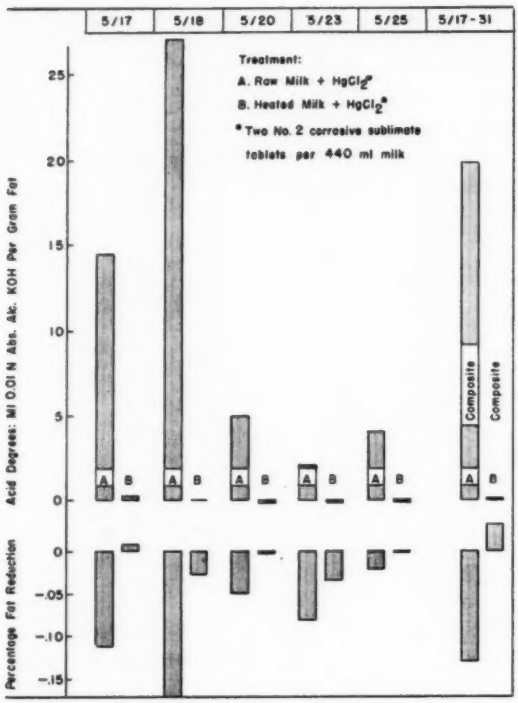

FIG. 2. Increase in free fatty acids compared with Babcock fat test reduction in five single preserved raw samples and five single preserved pasteurized samples and the composites of these samples during a 15-day period (1955).

In the first and second experiments the difference between the average of the butterfat tests of the fresh aliquots of the composites and the test of the respective composite represents the reduction in the butterfat test during the compositing period. Similarly, the difference between the average of the free fatty acid degrees of the fresh aliquots and the free fatty acid degrees of the respective composites represents the increase in free fatty acid degrees during the compositing period (Figures 1 and 2). In the second experiment the difference of the fat test of each fresh aliquot and of the aliquot at the end of the compositing period represents the fat test reduction occurring during the respective portion of the compositing period. Free fatty acid increases were determined for the same periods.

The Babcock test bottles used were especially selected for an accuracy of $\pm 0.05\%$, or smaller variation. In most instances the same glassware was used for each particular portion of the sample throughout the experiment in order to minimize any variation in the accuracy of the glassware.

The Babcock testing procedure is that described in *Official Methods of Analysis (1)*. The composites were warmed to 95° to 100° F. during a 30-minute period and recooled to 60°-65° F. prior to pipetting.

For determining free fatty acids the following modification of the method described by Shotwell *et al. (9)* was used: *(a)* 50 ml. of the milk were measured into a 200-ml. Erlenmeyer flask and the pH was adjusted to 2.0 with 1 *N* H_2SO_4; *(b)* the acidified milk was shaken vigorously with 16 ml. of 95% ethanol for 30 seconds and allowed to stand for 5 minutes; *(c)* 24 ml. of ethyl ether and 32 ml. of petroleum ether were added and the mixture was shaken for 40 seconds; *(d)* the mixture was transferred to a 240-ml. centrifuge tube and centrifuged for 10 minutes at 2,000 r.p.m. in a No. 2 International Centrifuge; *(e)* in the first experiment (Figure 1) most of the ether layer was siphoned into a dried, preweighed 125-ml. Erlenmeyer flask, and in the second experiment (Figure 2) most of the ether layer was decanted and 35-ml. portions were pipetted into the preweighed flasks; *(f)* the extracted fat was freed from solvents by using a 90° C. hot plate until bubbling ceased, with the last traces of solvent being removed by placing the fat in a 60° C. vacuum oven at 25 in. vacuum for 10 minutes; and *(g)* the flask was cooled in a desiccator and reweighed to determine the amount of fat. The free fatty acid content was determined by dissolving the fat in the flask by adding 5 ml. absolute ethanol and 15 ml. of petroleum ether. Ten drops of an absolute alcoholic phenolphthalein solution (1%) were added, and the solution was titrated from a 5-ml. microburette to the first definite color change with 0.01 *N* KOH in absolute ethanol. Results of the titration were expressed in terms of acid degrees: milliliters of 0.01 *N* base needed to titrate 1 g. fat. Blank values for the reagents were subtracted from the values obtained in titrating the dissolved fat.

The decrease in the pH values of ten paired composites of unpreserved and preserved milks was determined. Ten daily samples of milk were obtained at random from 10-gal. cans of milk from the College dairy for a period of 5 days. The preserved composites were prepared by dissolving two corrosive sublimate tablets in 440 ml. of each of the daily samples of milk. Each day 50 ml. of the paired unpreserved and preserved samples were placed in respective composite sample bottles and stored at 35° to 38° F. The pH value of each fresh aliquot was determined and on the seventh day after the first portions for the composites were obtained, the pH values of the composites were determined. The unpreserved samples were tasted at the end of the 7-day period by two experienced members of the staff. The odor of the preserved samples was noted.

RESULTS AND DISCUSSION

The data indicate an apparent activation of lipase by mercuric chloride. In the first experiment (Figure 1) the increases in free fatty acid degrees in the preserved raw milk composites at the end of the compositing and holding period of 7 to 8 days were: 19.39, 25.51, 12.78, and 8.97. The respective increase in free fatty acid degrees in the nonpreserved composites were: 0.67, 2.60, 0.38, and

0.33; for the respective composites of preserved pasteurized milk the increase in free fatty acid degrees were: not run, –0.05, –0.15, and –0.09. In the second experiment (Figure 2) the increase in free fatty acid degrees in the preserved raw milk composite at the end of the compositing and holding period of 15 days was 19.85 and in the corresponding preserved composite of pasteurized milk the increase was 0.15 acid degrees. The extensive hydrolysis of butterfat observed in the preserved raw milk composites appears to be brought about by the combined action of mercuric chloride and lipase. The observed increases in free fatty acid degrees in the presence of lipase alone were small in comparison, and when the milk was heated to inactivate the lipase prior to the addition of mercuric chloride the free fatty acid changes were so small as to be in the realm of experimental error. The addition of 20 p.p.m. of copper in the form of copper sulfate produced a slightly lower increase in free fatty acids and also a slightly smaller decrease in the fat tests (Figure 1).

In the second experiment (Figure 2) the individual samples from which the composites were prepared were tested for increase in free fatty acids and decrease in butterfat at the same time the composites were tested. The preserved raw aliquots showed large increases in free fatty acids and corresponding reductions in the fat tests, whereas the preserved pasteurized milk aliquots showed very small changes.

Visible evidence of fat hydrolysis was observed by the development of foam during the mixing of preserved raw milk composites in preparation for the Babcock test. No foaming was observed during the mixing of preserved pasteurized milk composites. Roahen and Sommer *(8)* found that lipolysis was greatly reduced when the pH of the milk was lowered from normal (6.6) to 6.25 and was markedly increased when the pH was raised from 6.6 to 8.90. In this experiment the addition of two No. 2 corrosive sublimate ($HgCl_2$) tablets to 440 ml. of fresh milk lowered the pH approximately 0.20 units. During a 7-day compositing and holding period the pH was still further decreased, approximately 0.2-0.3 unit. The immediate lowering of the pH by the addition of the preservative tablet would lead one to expect a decreased rate of lipolysis. However, this does not appear to be the case. Even though the pH of the unpreserved aliquots was higher and presumably more favorable to lipolytic activity than that of the preserved aliquots, there was less lowering of the pH (ca.0.04) during the compositing period and therefore presumably less butterfat hydrolysis. Only one of the 10 unpreserved composites was definitely rancid to taste, and three other composites were judged as very slightly rancid. All of the preserved composites had a rancid odor.

By statistically applying the observed $L.S.D._{.05} = 0.015$ (least significant difference) to the means of the decreases in fat percentage *(7)*, the following composites were found to be significantly different from the unpreserved raw milk composites: $HgCl_2$–preserved raw milk composite, $HgCl_2$ plus Cu–preserved raw milk composite, and $HgCl_2$–preserved pasteurized milk. Also, by applying the observed $L.S.D._{.05} = 5.252$ to the means of the free fatty acid increases, the following treatments were shown to be significantly different from

the unpreserved raw milk composites: $HgCl_2$–preserved raw milk and $HgCl_2$ plus Cu–preserved raw milk. The $HgCl_2$–preserved pasteurized milk composites were not significantly different from the unpreserved raw milk composites.

SUMMARY

Reductions in the fat tests of milk composites appear to be due to lipolytic hydrolysis. Mercuric chloride (in corrosive sublimate tablets) appears to increase lipolytic hydrolysis of butterfat. Twenty p.p.m. Cu, as copper sulfate, in the presence of mercuric chloride, slightly reduces the activating effect of $HgCl_2$. Heating the fresh milk to 160° F. for 30 to 60 seconds effectively prevents fat hydrolysis in composite samples preserved with mercuric chloride.

ACKNOWLEDGMENT

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VISCOSITY AND FLOCCULATION OF HEATED β-LACTOGLOBULIN SOLUTIONS: EFFECT OF CALCIUM CONCENTRATION AND pH

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A full knowledge of the effect of heat on milk is important for the understanding and control of the physical properties of heat-processed milk. The factors involved in the instability of milk to heat, including the time of heating and the influence of various salts, have been studied intensively *(17)*. Since milk is a complex substance, it appears desirable to amplify and interpret the data accumulated on it by a supplemental study of its individual components. Milk proteins, because of their lability, concentration, and diverse and numerous reactive groupings, deserve first consideration. It is expected that the proteins in heated milk will interact with other components of milk. It is important, therefore, to study the interaction of the various components.

This paper describes the interaction of two important components of milk, β-lactoglobulin and calcium, when heated within a range of calcium concentrations and pH values. The β-lactoglobulin has also been modified with a number of reagents in order to obtain information about the nature of the calcium effect. Light transmission, viscosity, gelling, and precipitation have been determined on the heated solutions. The results obtained are discussed in relation to the physical changes observed when milk itself is heated.

PROCEDURE

MATERIALS

β-lactoglobulin. This protein was prepared from raw milk by the method of Palmer *(12)* and recrystallized once. It was electrophoretically homogeneous at pH 8.6. One preparation was dried in the frozen state after dialysis against dilute ethanolamine (about 0.2 ml. per 10 l.) at pH 7.0. A second preparation was dried in the frozen state in the crystalline, isoelectric condition. This material was compact and easier to weigh than the fluffy material obtained when the solution at pH 7.0 was dried; when used, it was adjusted to pH 7.0 with ethanolamine to be comparable with the first preparation. Other pH adjustments of both preparations were made with HCl and NaOH. The dry preparations were stored in a desiccator at a relative humidity such that the moisture content was 10.0%. The concentrations given in the results are for the moisture-free product.

For some experiments the β-lactoglobulin was modified, particularly with reagents affecting the amino groups. The following reagents were used in the manner indicated.

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a) Formaldehyde. To 10.0 ml. of 4.5% β-lactoglobulin at pH 7.7 were added 3.8 ml. of 36.6% formaldehyde, reducing the pH to 5.9. The mixture was held 2 or 18 hours at 25° C. and finally dialyzed for 18 hours against water to remove the unreacted formaldehyde. The pH was raised to 7.5 and suitably diluted for the experiments with calcium. An estimation of the isoelectric point of heated 1.8% β-lactoglobulin solutions by adjustment of the pH to maximum precipitation showed that untreated β-lactoglobulin precipitated at pH 5.5, close to the expected pH of 5.2; the formaldehyde-treated solution precipitated at pH 5.0.

b) Nitrous acid. 0.9 g. of β-lactoglobulin was dissolved in 12.5 ml. 0.5 *M* acetate buffer, pH 4.3. To this were added 12.5 ml. of 2 *M* sodium nitrite. After the reaction had proceeded for 45 minutes at 25° C., the mixture was dialyzed against water for 18 hours. The β-lactoglobulin was precipitated from this solution with 3.2 *M* ammonium sulfate and dialyzed again. The solution was finally adjusted to pH 7.5 for the calcium experiments. A dry-weight determination established that no protein was lost in this procedure. An isoelectric point estimated from the maximum precipitation of a heated solution was at pH 4.7.

Methods

pH values. The pH values of the β-lactoglobulin solutions with all reagents present were measured at 25° C., before and after heating. The pH values at and above 7.0 dropped slightly on heating, sometimes several tenths of a unit. Below pH 7.0 the pH values rose several tenths on heating. The pH values after heating are given in the data.

Light transmission. Light transmission was measured in a Beckmann model B spectrophotometer at 600 mμ in the same tubes in which the solutions were heated.

Heating. The β-lactoglobulin solutions were heated in a constant-level water bath at 90° C., in 18 × 150-mm. Pyrex rimless test tubes. Small flanged test tubes containing ice water were placed in the top of the large tubes to reduce the loss of water vapor by evaporation. The volume of solution heated was 6.0 ml. and the time of heating, 30 minutes. In occasional experiments the time of heating was extended to 60 minutes, but no further changes were observed. This is to be expected from the rapid rate at which β-lactoglobulin is denatured at this temperature *(9, 10)*.

Viscosity. The viscosity was measured in a Bingham-type viscometer *(3)* at pressures of 15 to 50 cm. of water and at a temperature of 30° C. With water in the viscometer the product of the pressure (centimeters of water) and the time (seconds) was 517. The volume between marks was 2.97 ml., the length of the capillary 10.5 cm., and the radius of the capillary, calculated *(3)* from the flow time of water at 30° C., was 0.0335 cm. The air pressure was regulated with a Nullmatic[2] regulator and measured with a water manometer. The pressure was applied somewhat as described by Bingham *(2)*. The measurements were

[2] Mention of products does not imply endorsement of the U. S. Department of Agriculture or recommendation over any other products of a similar nature not mentioned.

made with the solution flowing upward or downward and a correction was made for the lack of symmetry (pressure head) *(3)* of the two arms of the viscometer. The readings recorded are averages of up and down readings at three different pressures. Absolute viscosities can be obtained with this viscometer, but the data in this report are expressed as viscosities relative to water at the same temperature. With the solutions studied in the present report the pressure-time product was always a constant, with a precision of $\pm 0.5\%$, and there was no evidence for structural viscosity.

RESULTS

A series of experiments was performed in which calcium chloride concentration and the pH were varied in 0.9 and 1.8% concentrations of β-lactoglobulin. The relative viscosity of unheated β-lactoglobulin solutions containing no calcium is 1.05 and 1.09 for the 0.9 and 1.8% concentrations, respectively, at pH 7.4. These values are increased slightly to 1.07 and 1.18, respectively, on heating. The concentrations of calcium used in this study have a negligible effect on the viscosity of unheated β-lactoglobulin. When calcium is present in heated solutions of β-lactoglobulin, however, marked changes in the viscosity are obtained. The determining effect of pH on the viscosity is shown in Figure 1, A (1.8% β-lactoglobulin) and B (0.9% β-lactoglobulin), with calcium concentrations up to those required for precipitation. The final viscosity increase with the high calcium concentrations is followed abruptly by gelling and the formation of a flocculent precipitate when the gel is disturbed.

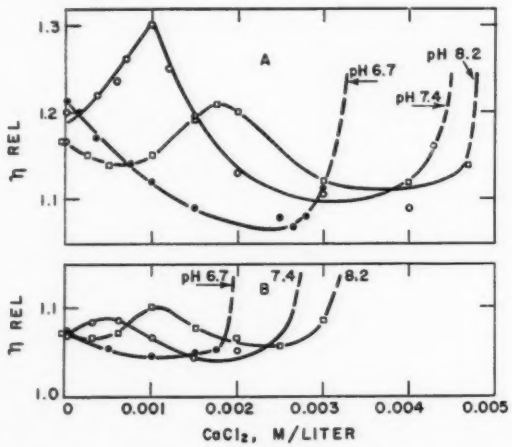

FIG. 1. The influence of calcium concentration and pH on the viscosity of β-lactoglobulin solutions heated for 30 minutes at 90° C. The calcium, added as calcium chloride, is present when the β-lactoglobulin is heated. The termination of the dashed lines is placed at calcium concentrations at which precipitation occurs and does not represent a viscosity value.

A. Viscosities of solutions containing 1.8% β-lactoglobulin at the pH values indicated.
B. Viscosities of solutions containing 0.9% β-lactoglobulin at the pH values indicated.

The heated solutions of β-lactoglobulin without calcium are clear, above pH 7, whereas below this pH they are increasingly opalescent as the isoelectric point (pH 5.2) is approached. When β-lactoglobulin solutions containing calcium are heated, those that show initial viscosity increases are slightly opalescent (absorbance readings less than 0.1 at 600 mμ). Solutions in which the viscosity decreases, i.e., "thinning" occurs, are white, and layers less than 1 mm. in thickness are very opalescent (absorbance readings at 600 mμ are greater than 2 and have no significance). Study of these solutions in the ultracentrifuge has shown that the protein in the white, "thin" solution was in a more aggregated condition, that is, it sedimented more readily, than in the clear, more viscous solutions.

Studies of the pH region between 7.3 and 6.7 have shown a gradual drop in the viscosity peak with a shift in the peak to lower concentrations of calcium. Experiments performed at pH 6.2 with a full range of calcium concentrations gave a more abrupt drop in the viscosity than at pH 6.7, and precipitation at a lower concentration of calcium. If calcium is added to solutions of β-lactoglobulin after heating, the solutions do not become white nor does the viscosity decrease.

The sharp dependence of the viscosity peak on pH suggested that a group dissociating in this pH region might be involved in a reaction with calcium. The α-amino group, with a dissociation pK of 7.8 *(16)*, appeared to be a possibility. The β-lactoglobulin contains four of these groups *(5)*. To obtain information on this point, β-lactoglobulin was treated with α-amino reagents (described under *Materials*), and the effect of calcium on the treated protein was determined.

Formaldehyde treatment (2 or 18 hours) had a striking influence on the reactivity of calcium in heated solutions of the treated protein. Experiments performed at pH 7.5 with 1.8% protein showed no viscosity peak; there was a slight drop in relative viscosity from 1.25 with no calcium to 1.12 with 0.006 *M* calcium. With larger concentrations of calcium the viscosity increased, and a precipitate was obtained with 0.0083 *M* calcium.

Nelson *(11)* has observed the stabilizing influence of small concentrations of formaldehyde on heat-treated milks. To approximate the conditions he used, 0.26 ml. of 36.6% formaldehyde was added to 25 ml. of 3.6% β-lactoglobulin. After 2 hours the pH was adjusted to 6.7 and the effect of calcium on a 1.8% solution was studied. With 0.005 *M* calcium the solution was opalescent, but no precipitate was obtained. At a concentration of 0.0067 *M* calcium a precipitate was obtained, a concentration of calcium over twice that required for precipitation of the untreated protein.

Parallel results were obtained on β-lactoglobulin treated with nitrous acid. A 1.8% solution of treated protein at pH 7.5 showed no viscosity peak with calcium. The viscosity of the solution with no calcium was 1.16, and about a 3% decrease in viscosity occurred in going to a concentration of 0.005 *M* calcium. A viscosity increase occurred above 0.007 *M* calcium and a precipitate was obtained at 0.01 *M* calcium.

In view of the possible role of amino groups glycine was added to give a concentration of 0.1 *M* to 1.8% β-lactoglobulin at pH 7.5 to see whether it would

influence the calcium reaction. There was no influence on the precipitation with 0.005 *M* calcium, and the usual viscosity peak was obtained with 0.001 *M* calcium.

The effect of lactose was determined at concentration of 5% in the 1.8% β-lactoglobulin solution at pH 7.5 with 0.003 *M* calcium. There was no apparent influence on the rate at which the solution turned white when heated, and the final viscosity could be estimated from addition of the specific viscosity of the components. Although it is well known that carbohydrates *(1, 7)* and other compounds with a large fraction of hydroxyl groups *(15)* inhibit denaturation rates, apparently the concentration of lactose present in milk is not high enough to have an appreciable effect.

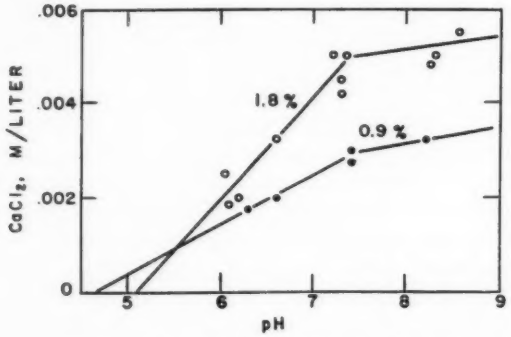

FIG. 2. The concentrations of calcium and pH values required for maximum precipitation of heated β-lactoglobulin solutions. Data are shown for 0.9 and 1.8% β-lactoglobulin solutions.

The relationship between the pH and the calcium concentration required to give a precipitate of heated β-lactoglobulin is shown in Figure 2. These results were obtained by heating a series of mixtures in the neighborhood of the precipitation point. White, gel-like precipitates were obtained, which on agitation gave "curds and whey." Maximum precipitation was evident from a water-clear whey. Actually, maximum precipitation occurs within a narrow range of calcium concentrations. A solution at pH 7.5 with 0.005 *M* calcium, which visually was maximally precipitated, was centrifuged at 2,500 × G for 10 minutes. The protein in the supernatant fluid was estimated from the nitrogen content, and 97.7% of the protein was in the precipitate. With a concentration of 0.0045 *M* calcium the supernatant fluid was slightly opalescent, but 90.0% of the protein was in the precipitate. With a concentration of 0.0040 *M* calcium a precipitate was evident when the heated solution was centrifuged, but a satisfactory separation could not be made. Probably incipient precipitates of this type account for the viscosity increases with concentrations of calcium slightly less than those required for precipitation (See Figure 1). There is considerable scatter of the points in Figure 2 because of the sharp dependence on pH and calcium concentration, but a consistent picture is obtained relating these variables. The 1.8% β-lactoglobulin solution consistently requires somewhat less than twice as much

calcium for precipitation as the 0.9% solution. The amount of calcium required for precipitation of β-lactoglobulin is proportionately greater as the pH of the solution increases above the isoelectric pH of 5.2 for β-lactoglobulin. Beyond pH 7, however, the requirement diminishes considerably.

DISCUSSION

The sharp dependence of calcium precipitation of heated β-lactoglobulin on the pH is of considerable interest and may be of importance for understanding the effect of heat on milk. A recent paper by Pyne and McHenry *(13)* on the heat coagulation of different milk samples established that the lower the effective calcium concentration, the longer the heating period required for coagulation. It was further established that all heated samples showed a drop in pH, and this was greatest (as much as one unit) for the samples that required the longest heating for coagulation. It can be assumed that if the pH had not dropped, some of these milks would have remained stable indefinitely. The authors discussed the key role of the pH on heat coagulation but the mechanism of the effect could only be surmised. The present studies with β-lactoglobulin show that the pH directly determines the protein precipitation at a given concentration of calcium, and presumably this will be true of other proteins also. A small drop in pH, probably less than 0.1 unit, is sufficient to change the system from a heat-stable to a heat-unstable condition.

In general, values for ionizable calcium and magnesium in milk *(4, 6, 13)* are at least 0.003 *M*, more than enough to precipitate β-lactoglobulin when milk is heated. Ramsdell and Whittier *(11)*, however, have observed that in milk heated to 100° C. for 10 minutes, although the whey proteins were denatured, they were not aggregated to the extent that they were sedimented with the casein in an ultracentrifuge. The β-lactoglobulin may be held in solution by other components of milk.

Probably the major sites of calcium binding on the β-lactoglobulin molecule are the negatively charged carboxyl groups; the extent of binding at these sites is determined, however, by the net negative charge, that is, binding increases as the pH is raised. The need for heat to bring about the precipitation of β-lactoglobulin by calcium shows, however, that the calcium reaction is not strictly electrostatic. The binding of the positive calcium ion at the negative carboxyl sites would, with proteins at a pH above the isoelectric point, lead to a reduction in the negative charge on the molecule. The proper concentration of calcium would bring the protein to the isoelectric condition where the net electrical charge is zero and isoelectric precipitation occurs. The data presented in Figure 2 support this interpretation of calcium precipitation. As the isoelectric point is approached, that is, as the net negative charge approaches zero, the amount of calcium required for precipitation decreases proportionately. The greater calcium concentration required for the precipitation of casein (unpublished studies) than for β-lactoglobulin, at a given pH, also supports this interpretation. The isoelectric point of casein is at pH 4.7, considerably lower than the pH 5.2 of β-lactoglobulin.

Although binding of calcium at carboxyl groups of β-lactoglobulin and reduction of the negative charge seem to explain calcium precipitation, other groups also appear to assist in the binding. The viscosity data with low calcium concentrations (0.001 *M*) show a high viscosity at pH 7.4 and a low viscosity at pH 6.7. This suggested that a group dissociating in this pH region might be participating. The α-amino group with a dissociating pK of 7.8 *(16)* fills this requirement. At pH 7.8 half of the amino groups would be in the free form, presumably the form assisting in the binding of calcium, whereas at pH 6.7 most of these groups would occur as the positively charged ion, apparently unreactive with calcium. The disappearance of the viscosity peak when β-lactoglobulin is treated with formaldehyde or nitrous acid lends support to this interpretation. Klotz *(8)* has reported data indicating that α-amino acids bind magnesium, and presumably calcium, more strongly than the corresponding aliphatic acid, which is further evidence for the probable participation of the amino groups of proteins in calcium binding.

The greater calcium requirement for precipitation of β-lactoglobulin after treatment with formaldehyde or nitrous acid can be interpreted in terms of net electrical charge on the protein. By altering the amino groups (ε-amino groups of lysine as well as α-amino groups) so that they are no longer positively charged, the net negative charge is increased, and the amount of calcium required to neutralize these groups, and thereby bring about precipitation, is increased correspondingly. The lowering of the isoelectric point (0.5 to 0.8 pH unit) by the chemical treatment and the magnitude of the pH effect shown in Figure 2 (about 0.0015 *M* calcium per pH unit) indicate, however, that the drop in isoelectric point alone does not quantitatively account for the increased amount of calcium required for precipitation.

Nelson *(11)* has found that small amounts of formaldehyde increase the heat stability of milk. Heat coagulation could be obtained by adding calcium to the formaldehyde-treated milk. The present studies indicate that the formaldehyde intervenes directly, by altering the charge on the proteins, in the amount of calcium required for precipitation of the milk proteins.

SUMMARY

The effect of calcium concentration, pH, and heat on the physical properties of β-lactoglobulin solutions was investigated. Viscosity, light transmission, and precipitation were measured. The dependence of viscosity on pH and the effect of certain α-amino group reagents suggested that α-amino groups assist in the binding of calcium ions. Precipitation in calcium-containing heated β-lactoglobulin solutions was sharply dependent on pH; the lower the pH, the smaller the concentration of calcium required. Calcium appears to function in large part to bring about an isoelectric precipitation by neutralizing negatively charged groups. The effects of heat on β-lactoglobulin solutions are discussed in relation to the heat stability of milk.

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DETERMINATION OF XANTHINE OXIDASE IN MILK WITH TRIPHENYL TETRAZOLIUM CHLORIDE

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Xanthine oxidase in crude biological preparations is frequently determined by the methylene blue decolorizing technique. Other more elegant methods are available for purified preparations of soluble xanthine oxidase *(5)*. The methylene blue technique requires the exclusion of oxygen to prevent the spontaneous reoxidation of the reduced, colorless dye. Hence, Thunberg tubes or some similar arrangement must be used to remove the oxygen. Triphenyl tetrazolium chloride (TTC) appeared to be more advantageous for determining xanthine oxidase since its red, reduced form is stable to oxygen. This substance has been used to determine xanthine oxidase in liver tissue *(6)* and in insects *(1)*. In both these studies the reaction was performed in evacuated tubes. This compound has been used also for the determination of succinic dehydrogenase, and oxygen was found not to interfere seriously *(4)*. A study of the use of TTC for xanthine oxidase in milk has shown that although the reduced compound is stable in air, the enzyme reaction is inhibited by oxygen presumably by competition with the TTC. A simplified technique has been developed, however, in which the enzymatic reaction is carried out in a system freed of air with bubbling nitrogen gas, and subsequent operations are performed in the air. The influence of TTC and substrate concentrations and other factors have also been investigated.

REAGENTS

Triphenyl tetrazolium chloride (TTC), 0.05 *M* 334.5 mg. is dissolved in 20.0 ml. of 0.1 *M* sodium phosphate buffer, pH 7.5.

Xanthine, 0.005 *M*. A 0.05 *M* solution is prepared by dissolving 380.0 mg. in 50.0 ml. of 0.05 *N* NaOH. This stock solution is diluted daily to the required 0.005 *M* concentration.

Phosphate buffer, 0.5 *M*, pH 7.5. Prepared by dissolving 29.1 g. $Na_2HPO_4 \cdot 7\ H_2O$ and 2.28 g. $NaH_2PO_4 \cdot H_2O$ in water and making the final volume 250 ml.

Acetic acid, glacial, C. P.

PROCEDURE

The enzyme xanthine oxidase activates certain hydrogen atoms on xanthine and on a variety of purine and aldehyde substrates with a concurrent reduction of oxygen or of a number of redox compounds. In the present method of determining xanthine oxidase, TTC is reduced by enzyme action to a red product

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which is extracted from the reaction mixture with toluene after acidification with acetic acid. The color in the toluene solution is read in a Beckman type B spectrophotometer[2] in 150 × 18 mm. test tubes at 485 mμ. The color equivalent of the reduced TTC was obtained by reducing dilute solutions of TTC with sodium hydrosulfite and extracting the color with toluene exactly as in the regular assay procedure. The results were not as reproducible as desired, but an approximate absorbance of 0.36 was obtained with 3×10^{-7} moles (0.6 ml. of 1:100 dilution of 0.05 *M* TTC) of reduced TTC. If this absorption is corrected for dilution in the toluene, the absorbance is 1.08 for 3×10^{-7} moles (100 γ) of TTC in 4.0 ml. of toluene. This value cannot be compared with other published values *(1, 4, 6)* because of incomplete description of the optical measurements.

One unit of xanthine oxidase activity is defined as follows: an absorbance of 0.036, equivalent to approximately 0.3×10^{-7} moles of reduced TTC, produced by the enzyme under the specified test conditions.

Determination of xanthine oxidase in milk. The enzyme reaction is performed in a water bath at 30° C. in 125 × 15 mm. test tubes. Two determinations are run in parallel with the following reagents present at the start: 1.0 ml. of 0.5 *M* sodium phosphate buffer, pH 7.5; 0.2 ml. xanthine solution; and water (1.5 ml. in one tube and 1.0 ml. in the other). The bubbling of commercial nitrogen gas into this mixture is begun, through small capillary tubes extending to the bottom of the test tubes, at a rate such that it is impossible to count the bubbles. The capillary tubes are grooved at the end to permit easy escape of the gas. By the use of a manifold as many as six test tubes can be in use simultaneously. The bubbling is continued for 5 minutes and then 0.5 ml. and 1.0 ml. of diluted milk (skim or whole milk 1:5) or cream (1:20) is added to the two tubes. If frothing occurs, it is controlled with a drop of antifoam (5 to 10 mg. Foamrex in 10 ml. water). Bubbling is continued for another 5 minutes. During this period the xanthine oxidase assists enzymatically in removing the last traces of oxygen. Finally, 0.2 ml. of TTC solution is added and the reaction is continued for an accurately timed 10.0 minutes. The final volume is 3.4 ml. The reaction is stopped with the addition of 5.0 ml. of concentrated acetic acid to each tube, and bubbling of the nitrogen is stopped. The capillaries are left in their respective test tubes and used to agitate the 4.0 ml. of toluene, which is added next. Finally, the tubes are stoppered, shaken vigorously, and centrifuged. The red reduced TTC is extracted into the toluene. Two ml. of the colored toluene solution are removed and diluted with 4.0 ml. of toluene. Six ml. of solution are required for the spectrophotometer tube. The colors are read in 150 × 18 mm. test tubes at 485 mμ.

DISCUSSION

The procedure described for the determination of xanthine oxidase in milk was developed after study of a number of factors that are critical for enzyme reactions including concentration of both the substrates and the reagent.

[2] Mention of products does not imply endorsement of the U. S. Department of Agriculture or recommendation over any other products of a similar nature not mentioned.

The optimal concentration of xanthine in the reaction mixture is 0.000088 to 0.00037 mole per liter (0.06 to 0.25 ml. of a 0.005 *M* solution), as shown in Figure 1. At higher concentrations inhibition occurs; for example, a concentration (0.000588 *M*) twice that used in the assay inhibits the reaction by 15%. Inhibition by excess substrate is characteristic of this enzyme *(2, 5)*. Smaller concentrations of xanthine are inadequate for maximum activity. The substrate requirement was studied with an amount of enzyme that gave a maximum

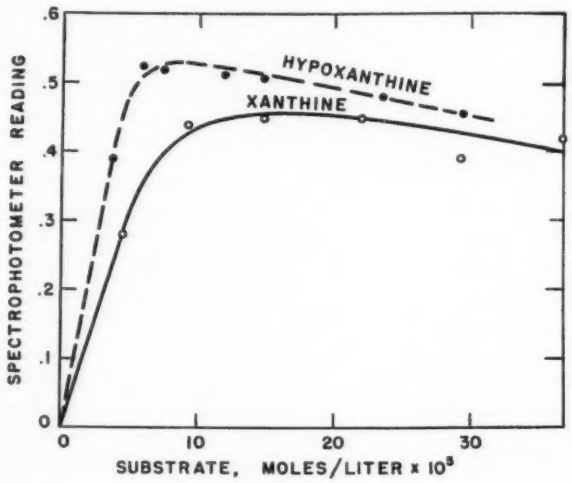

FIG. 1. Effect of xanthine and hypoxanthine concentration on xanthine oxidase activity.

absorbance reading of 0.45 with xanthine. Readings should not exceed 0.6 for the substrate concentration routinely used (0.2 ml. of 0.005 *M*), or the concentration will begin to limit the activity, and the readings and amount of enzyme will not be proportional.

The concentration of TTC required for maximum reactivity is surprisingly high, over a tenfold range of xanthine concentrations, as shown in Figure 2. Methylene blue behaves similarly, but only if the concentration of substrate is high *(2)*. The same concentration dependence of TTC is found with hypoxanthine as the substrate. The concentration of 0.2 ml. of TTC used in the regular assay (final concentration 0.0029 *M*), although below the optimum, is justified since the concentration of TTC changes only about 5% during the reaction.

The pH must be maintained at 7.5 for maximum activity. If acidic undiluted milk samples are assayed, the effectiveness of pH maintenance must be determined. The stability of the xanthine oxidase in milk under the test conditions, particularly after the xanthine was introduced and before the TTC was added, was established by prolonging the bubbling time from 5 to 10 minutes. The results with both bubbling times were the same, indicating that the enzyme was stable during this period. The stability of the enzyme is evident since the amount

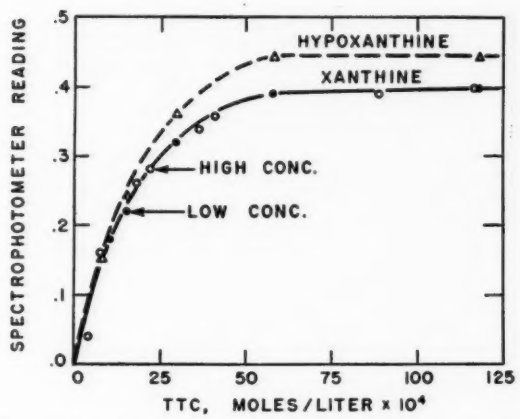

FIG. 2. Influence of triphenyl tetrazolium chloride (TTC) concentration on xanthine oxidase activity with hypoxanthine and two concentrations of xanthine. Concentrations in reaction mixture: xanthine 0.00182 (high) and 0.000182 (low) mole per liter; hypoxanthine 0.000059 mole per liter.

of TTC reduced is proportional to the time the enzyme acts on the TTC. The amount of TTC reduced is also proportional to the amount of enzyme. In routine assays two amounts of enzyme are used: 0.5 and 1.0 ml. of a suitable dilution as indicated in the procedure. This serves to establish the reproducibility of the assay. Also, if the 0.5-ml. value is lower than the 1.0-ml. value, the bubbling is likely to have been inadequate. If the 1.0-ml. value is low, the amount of enzyme used is too great (absorbance greater than 0.6). This should not occur if the dilutions of milk and cream indicated are used. Routine assays with this method are reproducible with less than $\pm 5\%$ deviation.

Some work was also done with hypoxanthine instead of xanthine, and neotetrazolium instead of TTC. The reaction rate with hypoxanthine was about 25% faster than with xanthine, as was expected from previous observations *(2, 5)*. Also, maximum reactivity occurred with more dilute solutions, as little as 0.00006 mole per liter being adequate (Figure 1). This difference between the two substrates has been observed recently with a purified xanthine oxidase *(5)*. Neotetrazolium was found to react with the xanthine oxidase of milk at about the same rate as TTC. This substitute reagent was tried since it had been reported to have more than ten times the reactivity of TTC with insect xanthine oxidase *(1)*, and it was hoped that its faster reaction might not have been inhibited by oxygen. It was found, however, that oxygen was inhibitory with it also.

Application of the procedure. The xanthine oxidase in raw market milk, as determined by these procedures, was found to be 140 units per milliliter for cream (separated by gravity, about 25% butterfat) and 25 units per milliliter for skimmilk. Cream from another source (25% butterfat) contained 210 units per milliliter.

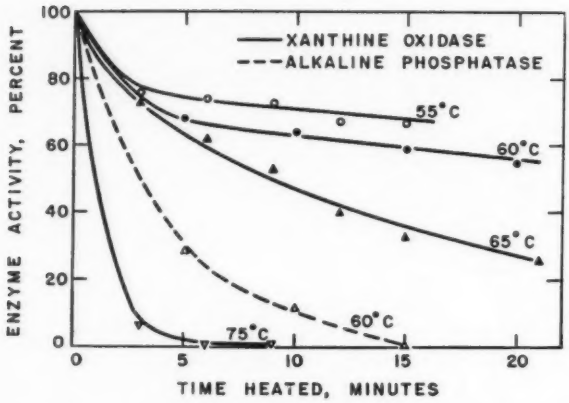

FIG. 3. Effect of heat on the xanthine oxidase and alkaline phosphatase in skimmilk. Alkaline phosphatase determined with phenyl phosphate as substrate in ethanolamine buffer at pH 9.6 *(7)*. The minutes heated do not include the time to come to temperature, which was 2 to 3 minutes.

The xanthine oxidase method has also been useful in characterizing the fat globule membrane and a related fraction in whey *(8)*.

Another application of the method is illustrated in Figure 3, which compares the decrease in the xanthine oxidase, and also in the alkaline phosphatase activity, of skimmilk when exposed to various elevated temperatures from 55 to 75° C. The decrease in alkaline phosphatase follows the usual pattern *(3)*, and the xanthine oxidase, although more resistant to heat than alkaline phosphatase, decreases similarly.

SUMMARY

A method is described for the determination of xanthine oxidase in milk with triphenyl tetrazolium chloride. The enzymatic reaction is performed with nitrogen gas bubbling through the mixture to avoid oxygen which is inhibitory. Subsequent steps, which include extraction of the color with toluene after acidification with acetic acid, are performed in air since the red, reduced form of triphenyl tetrazolium chloride is stable. The effects of concentration of substrate and triphenyl tetrazolium, and other factors are investigated. The method is used to show the destruction of xanthine oxidase in skimmilk at 55 to 75° C.

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THE FAT-GLOBULE MEMBRANE OF MILK: ALKALINE PHOSPHATASE AND XANTHINE OXIDASE IN SKIMMILK AND CREAM

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Recent studies of Morton *(11, 13)* show that the fat-globule membrane of milk has many properties in common with the enzyme-containing, lipoprotein complexes of animal tissues termed microsomes. The complex nature of the fat-globule membrane suggests that isolation methods involving pasteurization *(3)*, the use of low pH *(6)*, and lipide-solvents *(3, 6)* should be avoided to isolate this material in its native state. Further, by avoiding pasteurization, enzyme assays (xanthine oxidase and alkaline phosphatase in the present studies) can be used to follow the fractionation. In the present report the lipoprotein fraction from cream is compared with a similar fraction from skimmilk in behavior in the ultracentrifuge and in filtrability, chemical composition, and enzyme content.

METHODS

Alkaline phosphatase. Phenol phosphate is used as substrate and the assay is performed at pH 9.6 in ethanolamine buffer at 37° C. *(18)*. Enzyme activities are expressed in terms of optical absorbance in an 18 mm. diameter calibrated test tube at 660 mμ. An absorbance of 0.105 (10.5 units) is equivalent to the release of 1.0×10^{-7} moles of phenol.

Xanthine oxidase. The method is based on the ability of xanthine oxidase to catalyze the reduction of the colorless triphenyltetrazolium chloride to a red substance when xanthine or other suitable substrate is present. Details of the method are described elsewhere *(17)*. The formation of 3×10^{-7} moles of reduced triphenyltetrazolium under the conditions of the test is defined as 10 units of oxidase.

Determination of lipides by extraction with organic solvents. Five hundred to 800 mg. of freeze-dried solids were extracted twice with 40 ml. acetone and finally with 40 ml. ethyl ether. When it was desired to dissociate the lipoprotein *(10)*, the acetone extraction was followed by 40 ml. *n*-butanol and finally with ethyl ether.

Determination of nitrogen and protein. The nitrogen content was determined by the Nessler method. Comparative protein estimations were made routinely with the biuret reagent.

Determination of phosphorus. Samples were digested with sulfuric acid and hydrogen peroxide. Phosphorus was estimated with the ammonium molybdate reagent after reduction with ferrous sulfate.

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Ultracentrifuge. The Spinco preparative ultracentrifuge was used with No. 40 rotor and 13.5-ml. plastic tubes. In the experiments described, the ultracentrifuge was run for ½ hour at 40,000 r.p.m. (105,400 × G). All solutions were centrifuged first at 3,000 r.p.m. (approx. 2,500 × G) for 10 minutes to remove gross particles.

EXPERIMENTAL PROCEDURE

Preparation of lipoprotein membrane from cream. The procedure of Jenness and Palmer *(9)* was followed closely for preparation of the "membrane" by using unpasteurized cream obtained commercially, which contained 25% butterfat. The enzyme distribution in a typical experiment through successive washings and final churning of the cream is shown in Table 1. The enzyme in the

TABLE 1

Xanthine oxidase and phosphatase in cream, washed cream, and the buttermilk

	Xanthine oxidase	Alkaline phosphatase
	(units/1 ml.)	*(units/1 ml.)*
Cream[a]	51	123
1st washed cream	42	76
1st wash water	14	40
2nd washed cream	22	48
2nd wash water	14	20
3rd washed cream	16	35
3rd wash water	8.6	15
4th washed cream	9.1	30
4th wash water	7.5	5
Buttermilk	7.0	18

[a] All assays are calculated to the volume of the cream (14.0 l., obtained by dilution of 3.5 l. of 25% cream with water). The volumes of wash water were actually 11 l., volumes of the 4th cream 2.25 l., and the buttermilk obtained from this 1.12 l.

washed cream and the wash usually adds up to the enzyme in the previous cream, indicating that there is no serious destruction of the enzymes in this procedure. In this experiment, 15% of the phosphatase and 14% of the xanthine oxidase were recovered in the buttermilk. The losses of phosphatase into the water during the washing are considerably greater than those reported by Rimpila and Palmer *(16)*. This might be due to the chilling to which the starting cream had been subjected, since this increases the dissociation of the lipoprotein from the cream *(9)*. Aging of whole milk at low temperature has also been reported *(2)* to increase the portion of xanthine oxidase in skimmilk.

The proteins of the washed cream buttermilk were concentrated by precipitating with 3.2 *M* ammonium sulfate. The damp salt precipitates were stored at 7° C. The retention of activity in the salt cakes has been variable, but frequently the loss in activity was as little as 10% in 30 days. The preparations have also been dialyzed, which process in itself causes some loss in xanthine oxidase activity, and then dried from the frozen state. Freeze-drying caused, on the average, a 15% loss of the alkaline phosphatase but a 46% loss of xanthine oxidase. The

freeze-dried product contained approximately 5 units of xanthine oxidase and 15 units of alkaline phosphatase per 1 mg. of solids. A rapid additional loss in xanthine oxidase followed on storage of the dried material at 7° C., and activity was almost completely lost by the end of 30 days. Alkaline phosphatase, on the contrary, was quite stable.

The freeze-dried buttermilk preparations contained 60 to 70% of material that was extractable with acetone and ether. Others *(16)* have found with similar preparations that like amounts (56 to 78%) could be extracted with alcohol and ether. Much of this extracted fraction is probably butter oil. The phosphorus content of the fraction obtained by acetone and ether extraction was 0.25%, indicating that only 6% *(16)* of the extracted fraction is phospholipide. The residue, after acetone-ether extraction, contained 11.0% nitrogen and 0.53% phosphorus, values in agreement with previous reports *(6)* for the membrane component. Additional material (18%, containing 1.7% phosphorus) was extracted from the acetone-ether residue with *n*-butanol, a reagent which appears to be especially effective in dissociating lipoprotein *(10)*. The residue, after this treatment, contained 11.8% nitrogen and 0.24% phosphorus. Even when these extractions were performed at 5° C., a considerable loss in enzyme activity occurred, particularly of the xanthine oxidase. The alkaline phosphatase activity of this product was about 40 units per 1 mg. solids. The membrane fraction (microsomes) obtained by ultracentrifugation has been reported *(13)* to contain 1% nucleic acid phosphorus, equivalent to approximately 10% nucleic acid. By using the same analytical methods, less than 0.05% nucleic acid phosphorus was found in the present studies. Even if all the phosphorus (0.24%) in the solvent-extracted residue belonged to nucleic acid, this would be equivalent to only 2.4% nucleic acid. Further studies by Morton *(14)* have shown that microsomes from the milk of individual cows show considerable variation in nucleic acid content but that microsomes from bulk milk contain about 0.25% nucleic acid.

Preparation of lipoprotein from skimmilk. Approximately 60% of the alkaline phosphatase of whole milk is found in skimmilk *(7)*. The distribution of xanthine oxidase is roughly the same *(2)*. Assays for these enzymes served to guide the isolation of a fraction from skimmilk for comparison with the cream preparation. The method followed in part the purification of the alkaline phosphatase of milk *(20)*—precipitation of the casein with 1.38 *M* ammonium sulfate and separation of the whey fraction that precipitates with 2.3 *M* ammonium sulfate. This precipitate was stored as a damp salt cake, which was dissolved in water for further study. Alkaline phosphatase in this form is quite stable. Xanthine oxidase shows some loss on storage.

The pellets obtained on ultracentrifugation (see later) of solutions of the 2.3 *M* ammonium sulfate precipitates from the whey are more comparable to the cream preparations in levels of enzyme activities, particularly alkaline phosphatase (immune globulins, etc. present in the 2.3 *M* fraction remain in the supernatant fluid on ultracentrifugation), than is the starting material. Accordingly, the properties of the ultracentrifuge pellet of the 2.3 *M* whey precipitate are compared with the washed cream buttermilk fraction. The pellet contained ap-

proximately 20 units of alkaline phosphatase per 1 mg. freeze-dried solids. These whey pellets have shown the same relative enzyme stabilities as the cream preparations. Nine per cent of the pellet material dried from the frozen state was extracted with acetone and ether. Extraction of the residue with *n*-butanol (1.7% of the extract being phosphorus) removed an additional 18%. The residue after extraction contained 10.7% nitrogen and 0.24% phosphorus. This fraction, too, contained less than 0.05% nucleic acid phosphorus.

TABLE 2

Distribution of xanthine oxidase and alkaline phosphatase in cream, skimmilk, and fractions of the latter

	Xanthine oxidase	Alkaline phosphatase
	(units/1 ml.)	*(units/1 ml.)*
Cream[a]	143	400
Skimmilk[b]	23	88
Whey[c]	18	28
2.3 *M* precipitate[d]	10	22

[a] Obtained by gravity separation (approx. 25% fat). This cream was of a different source than the cream used in the buttermilk studies shown in Table 1. The xanthine oxidase activity was regularly lower.

[b] All assay values except the cream were calculated in terms of the volume of skimmilk. The relative volumes of skimmilk and cream were 6.5 : 1.

[c] Casein removed with 1.4 *M* ammonium sulfate.

[d] Precipitated from whey by raising the ammonium sulfate concentration to 2.3 *M*.

The data in Table 2 show that there was considerable loss of alkaline phosphatase when casein was removed from skimmilk with ammonium sulfate (1.4 *M*). NaCl (36 g. per 100 ml.) was equally detrimental. Some of the missing alkaline phosphatase was found in the casein fraction (about 25% of the total), but there was a destruction of phosphatase as well. Morton *(12)* found a number of other salts equally detrimental to the alkaline phosphatase. This behavior of alkaline phosphatase is strange, since in later stages of purification *(20)* ammonium sulfate is a satisfactory precipitant. Xanthine oxidase, on the other hand, was recovered almost quantitatively in this fractionation. About 60% of the alkaline phosphatase was recovered in the whey when rennin *(18, 20)* or pepsin *(12)* was used to remove the casein. In one experiment, crystalline pepsin was used (0.5 mg. per 250 ml. skimmilk at pH 6.5 and 35° C.) for this purpose, with the thought that only the clotting of casein would occur with no proteolysis of other proteins. The recovery of xanthine oxidase and alkaline phosphatase in the whey was 68% and 58%, respectively. On centrifuging this material, however, only 10% of the enzymes was obtained in the pellet. Presumably, the pepsin had caused some dissociation of the lipoprotein complex. Crude proteolytic enzyme preparations have frequently been used for this purpose in the purification of alkaline phosphatases *(20)* and xanthine oxidase *(1, 2, 8)*. Recent experiments have shown that precipitation of the casein with acetic acid at pH 4.7 was more satisfactory for recovery of the alkaline phosphatase in the whey than the use of ammonium sulfate. (Immediately after precipitation and filtration of the casein, the whey was adjusted to pH 7.0). Approximately 50% of the activity of both enzymes was recovered in the whey.

Ultracentrifugation of the lipoprotein from cream and skimmilk. The results obtained when the washed cream buttermilk fraction was subjected to ultracentrifugation for ½ hour at 40,000 r.p.m. (105,400 × G) are shown by the following typical example. The ammonium sulfate precipitate of the washed cream buttermilk was dispersed in water to a concentration of 0.26% protein. The solution contained 228 units of phosphatase and 60 units of xanthine oxidase per milligram of protein. Eleven and one-half ml. were placed in each tube. After centrifugation, a top milky fraction of 1.8 ml. was removed with a supported syringe and needle, the intermediate fraction of 9.7 ml. was decanted, and the pellet was dispersed in 2.6 ml. of 0.1 *M* ethanolamine buffer, pH 9.6 *(18)*. The distribution of enzymes is shown in Table 3. Considerably more of the xanthine

TABLE 3

Distribution of enzymes on centrifugation of the lipoprotein from cream and skimmilk

	Xanthine oxidase				Alkaline phosphatase		
	Vol. *(ml.)*	*(units/ 1 ml.)*	*(units/1 mg. protein)*	Recovery *(%)*	*(units/ 1 ml.)*	*(units/1 mg. protein)*	Recovery *(%)*
			Lipoprotein from cream				
Original	11.5	155	60	100	592	228	100
Top	1.8	7.5	—	7.6	717	—	19.0
Intermediate	9.7	23	5.2	12.8	222	148	31.6
Pellet	2.6	472	108	69.0	824	187	31.5
			Lipoprotein from skimmilk				
Original	11.5	130	9.2	100	239	17	100
Supernatant	11.5	95	—	73.0	155	—	64.8
Pellet	2.0	78	15.6	10.4	540	108	39.3

oxidase was obtained in the pellet than the alkaline phosphatase with some increase in purity of the former but not of the latter. Much of the top milky layer is probably unchurned cream or butterfat since, after drying, 95% of this top fraction was extracted with acetone and ether. The amount of this top fraction is small in terms of solids.

In some experiments the buttermilk fraction was prepared by washing the cream with magnesium chloride (0.1%). The magnesium salt had to be dialyzed from the final cream for churning to proceed satisfactorily. Some experiments suggested that the recovery of enzymes was improved, but in general the results were variable. In the ultracentrifuge it was regularly found, however, that material prepared in this way deposited considerably more of the enzymes in the pellet. The recovery of the alkaline phosphatase in the pellet was 65%; the xanthine oxidase recovery was 80%.

A typical ultracentrifuge experiment with the skimmilk lipoprotein fraction is illustrated also in Table 3. The fraction was concentrated by ammonium sulfate precipitation, and the solution contained 1.41% protein. With this material no top layer was obtained. In this instance the alkaline phosphatase recovery in the pellet was about the same as the buttermilk. Since the alkaline phosphatase protein was a small part of the total, this brings about an increase in the

purity to the approximate level of the washed cream buttermilk fraction. When the pellet was recentrifuged, over 80% of the alkaline phosphatase was found in the new pellet. Xanthine oxidase sedimented very poorly. On centrifuging acid whey, 67% alkaline phosphatase and 37% xanthine oxidase were recovered in the pellet—somewhat more than the amounts obtained when the 2.3 *M* ammonium sulfate whey fraction was centrifuged.

Filtration with Filter-Cel.[2] Treatment of the milk fractions with Filter-Cel provides information that may be used to characterize the different fractions. Filtration of the skimmilk lipoprotein preparation with 1% Filter-Cel mixed with it removed approximately 40% alkaline phosphatase and 20% xanthine oxidase. The removal of alkaline phosphatase by Filter-Cel is due to a rapid adsorption *(19, 20)*, and this is probably true also of xanthine oxidase. Use of the ultracentrifuge on Filter-Cel-treated solutions of the skimmilk fraction indicated that the alkaline phosphatase in the pellet fraction was decreased to 12%, compared with 39.3% before treatment (Table 3). There was little loss of xanthine oxidase on filtration and no reduction in the pellet fraction in subsequent ultracentrifugation. The ability of Filter-Cel to remove the enzymes appears to parallel the association of the enzymes with large, easily sedimentable complexes. It was noted in the pepsin whey experiment that not only were the enzymes poorly sedimented but also the solution could be filtered with 1% Filter-Cel with only a 10% reduction in the enzymatic activities. Filtration of the cream lipoprotein preparations with 1% Filter-Cel leads to a 90% removal of both enzyme activities, indicating that both enzymes are associated in these preparations with relatively large complexes. This is supported by the ultracentrifuge results.

DISCUSSION

The milk microsomes are about 100 mμ in cross section and contain a number of enzymes in addition to xanthine oxidase and alkaline phosphatase *(11)*. Among the other enzymes present in the milk microsome, Morton reported *(11, 13)* diaphorase, DPN-cytochrome C-reductase, and a hemochromogen, probably cytochrome C. Acid phosphatase, esterase, adenosine-5′-phosphatase and uricase also have been reported *(15)* in the microsome fraction from other tissues. The microsomes contain protein, phospholipide, and nucleic acid. They can be pictured as a parcel of enzymes cemented together by phospholipide and nucleic acid. Electrophoresis of a fat-globule membrane preparation has shown as many as three components *(5)*. Several stages of dissociation of this complex appear to be a definite possibility. Morton *(11, 13)* has summarized the evidence for his conclusion that the unique membrane component of the fat-globule is the complex microsome. Size, composition, and other properties support this conclusion. Morton concluded also *(14)* that the low nucleic acid content of this fraction in milk compared with the microsome fraction from the mammary gland probably results from the action of a milk enzyme.

[2] The mention of products does not imply endorsement or recommendation by the Department of Agriculture over other products of a similar nature not mentioned.

Formerly it was believed that the microsome was a unit substance. Recent evidence *(15)*, however, indicates that microsomes have a range of sizes and different distribution of enzymes. Each complex unit probably contains a number of enzyme proteins. By suitable dissociative methods, each enzyme protein, as well as other components, can be obtained in the free form and purified. The present studies show that xanthine oxidase and alkaline phosphatase activities are not parallel throughout the fractionation of the easily sedimented components, suggesting that these enzymes might occur in different complexes, or in different proportions in the same complex, with only slightly different physical properties. The preparations from ammonium sulfate and pepsin wheys indicate also that the alkaline phosphatase component is more strongly adsorbed to casein than is the xanthine oxidase component under a variety of conditions. An ultracentrifuge study *(13)* of skimmilk does not show this microsome fraction, for it is diluted out by the much larger amount of the casein complex. This study of the skimmilk fraction suggests that although the alkaline phosphatase occurs in the same form as in the cream fractions, xanthine oxidase occurs as a smaller, much less easily sedimented unit. Purification studies have indicated that the xanthine oxidase unit is much more easily dissociated *(1, 2, 8)* to give the free protein than is the alkaline phosphatase *(20)*. The association of these enzymes with the fat-globule membrane gives added interest to studies of their distribution between cream and skimmilk under the influence of temperature changes, agitation, etc.

Unfortunately, the enzymes cannot serve to follow the microsome fraction during the heat treatment of milk because of their inactivation. It is proposed to characterize this fraction by its easily sedimentable nature, as well as by some other property, such as the colored cytochrome C content, to distinguish it from casein. Sedimentation studies *(4)* of the fat-globule membrane, after extraction with lipide solvents, did not show any easily sedimentable components. A similar experiment in the present series showed that only 8% of the total nitrogen of an extracted preparation was obtained in a pellet fraction. This suggests that the phospholipide is the major cementing component of the membrane complex. Distribution of the phospholipide with various heat treatments of the membrane complex will be of interest.

SUMMARY

The fat-globule lipoprotein membrane of milk has been prepared from cream by washing and churning. Physical and chemical properties of this fraction have been correlated with xanthine oxidase and alkaline phosphatase activities. The membrane fraction has been compared with a related fraction obtained from skimmilk in behavior in the ultracentrifuge, in filtrability, chemical composition, and enzyme content.

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NOMENCLATURE OF THE PROTEINS OF BOVINE MILK

Report of the Committee on Milk Protein Nomenclature, Classification, and Methodology of the Manufacturing Section of A.D.S.A.

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Modern techniques of protein chemistry have shed a great deal of light on the nature and properties of the individual proteins of milk. The older empirically defined fractions have been shown to consist of mixtures, and several rather homogeneous proteins have been isolated and well characterized by the methods of electrophoresis, ultracentrifugation, immunology, and amino acid analysis *(3, 5, 6, 8, 10, 12, 18, 20, 21, 22, 27, 28)*. Thus the classical nomenclature of the milk proteins in terms of "casein," "lactalbumin," and "lactoglobulin" is no longer adequate. On the other hand, a complete transition to a new nomenclature in terms of the individual components is not possible as yet because a number of components remain to be isolated and characterized. In a situation of this kind it is natural that some confusion has arisen. It is the purpose of this report to attempt to clarify the nomenclature of milk proteins by presenting a summary of current "preferred" usage and by showing the relationship between the individual proteins that have been isolated and the classical fractions.

The eight reasonably pure and homogeneous proteins that have been isolated from bovine milk in appreciable quantity are listed in Table 1 with some of their distinguishing characteristics. Actually, probably none of the proteins included in this list is entirely homogeneous. The immune globulins which carry the antibodies of milk have been separated into two fractions on the basis of water solubility (eu- and pseudo-globulin). These two fractions are somewhat heterogeneous in both physical properties and biological specificity. There is evidence that β-lactoglobulin consists of two closely related kinds of molecules [designated β_1- and β_2-lactoglobulin *(1, 22)*]. Similarly, α-casein may be a mixture of two kinds of molecules. As further fractionation and purification of these proteins is accomplished in the future, changes in nomenclature will be necessary. Nevertheless, the nomenclature employed in Table 1 is in common usage at present; it is recommended that it be followed by dairy scientists and others dealing with milk proteins.

Quantitative electrophoresis by the moving boundary method has been used to analyze milk for the proportions of these individual proteins. The several electrophoretic peaks in such patterns have been identified with the various isolated proteins on the basis of mobility *(9, 12)*, and the composition is deter-

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TABLE 1

Individual proteins isolated from milk and some of their properties

Protein	Classical fraction from which isolated	Reference to preparation	Approximate per cent of milk proteins[a]	Mol. wt.	pI[b]	Mobility at pH 8.6[c]	Other distinctive characteristics
α-Casein	Casein	8	45-63	?	4.1	−6.7	1% Phosphorus
β-Casein	Casein	8	19-28	24,100[d]	4.5	−3.1	0.6% Phosphorus
γ-Casein	Casein	8	3-7	?	5.8-6.0	−2.0	0.11 % Phosphorus
β-Lactoglobulin[e, f]	Lactalbumin	10, 18	7-12	35,500	5.18	−5.1	1.1% Cysteine
α-Lactalbumin[e]	Lactalbumin	5, 6	2-5	15,100	4.1-4.8	−4.2[g]	7% Tryptophan
"Blood" serum albumin[h]	Lactalbumin	21	0.7-1.3	65,000	4.7	−6.7	Appears to be identical to albumin of bovine blood
Immune globulins:							
Euglobulin	Lactoglobulin	27	0.8-1.7	180,000[i]	6.0	−1.8	Carry antibodies. Present in high concentration in colostrum. Contain hexose and hexosamine.
Pseudoglobulin	Lactoglobulin	27	0.6-1.4	180,000[i]	5.6	−2.0 to −2.2	

[a] As determined by measurements of relative areas of electrophoretic components in casein (1.5%) and serum protein (2.0%) fractions at pH 8.6 and 0.1 ionic strength *(11, 23)*.

[b] Isoelectric point, i.e., pH of no electrophoretic migration.

[c] Electrophoretic mobility in units of 10^{-5} cm.^{2} volts^{-1} sec^{-1} obtained by the Tiselius moving boundary method at ~ 2° C. in veronal buffer at pH 8.6, ionic strength = 0.1, (0.02 *M* veronal, 0.1 *M* Na veronal).

[d] Value applies below 15° C. only.

[e] Prefixes α and β for α-lactalbumin and β-lactoglobulin were assigned on the basis that these proteins sediment at the same rates in the ultracentrifuge as peaks designated α- and β- for skimmilk by Pedersen *(20)*.

[f] β-Lactoglobulin appears to consist of two closely related components designated β_1 and β_2 lactoglobulin which differ slightly *(1, 22)*.

[g] Mobility −3.6 in the milk serum protein mixture *(9)*.

[h] Some of the data for this protein are for material actually isolated from blood.

[i] Protein not entirely homogeneous; molecular weight of major component.

TABLE 2

Protein analyses of skimmilk comparing newer and older nomenclature

Electrophoretic analysis[a]		Rowland procedure[b]	
(Protein)	*(g/100 ml.)*	*(Classical fraction)*	*(g/100 ml.)*
α-Casein	1.4-2.3		
β-Casein	0.5-1.0	Casein	2.2-3.5
γ-Casein	0.06-0.22		
		Total serum proteins	0.4-0.8
β-Lactoglobulin	0.20-0.42		
α-Lactalbumin	0.07-0.15	Lactalbumin fraction	0.15-0.40
"Blood" serum albumin	0.02-0.05		
Immune globulins	0.05-0.11	Lactoglobulin fraction	0.07-0.20
Other components	0.06-0.17	Proteose-peptone fraction	0.06-0.25

[a] Data from Rolleri *et al. (23)* based on 2-day composite samples from 28 cows of five breeds in lactation 2-6 months.
[b] Data from references 7, 11, 14, 17, 23, 25, 26.

mined by measurements of areas under the peaks. Results of such analyses of skimmilk are presented in Table 1. In Table 2 the electrophoretic analyses are compared with results obtained by a classical fractionation procedure. These quantitative electrophoretic analyses must be accepted with some reservation at present because it is not absolutely certain that each electrophoretic peak arises solely from the protein to which it is ascribed; another unrecognized protein or proteins may contribute to it. Furthermore, the ratios between peak areas may not correspond exactly to the ratios of protein concentrations because *(a)* the individual proteins differ slightly in refractive index increment, *(b)* interactions may occur between proteins and between proteins and buffer ions, and *(c)* concentration gradients of buffer ions may be superimposed on the concentration gradients of proteins. Nevertheless the electrophoretic analyses in Table 2 represent at least a first approximation of the protein composition of milk in terms of individual constituents.

In order to discuss the relationship of the individual proteins to the classical fractions, it is necessary to define the latter. For many years the proteins of skimmilk were divided into three principal fractions called casein, lactalbumin, and lactoglobulin, which were separated on the basis of differences in solubility *(4, 16)*. An analytical scheme described by Rowland *(24)* has been employed considerably in recent years to determine these fractions. Actually Rowland designated a fourth fraction as "proteose-peptone" consisting of the heat-stable milk serum proteins. In addition to these protein fractions of skimmilk, whole milk contains a lipoprotein fraction primarily associated with the fat globules and designated the fat globule "membrane" proteins *(15, 19)*. These fractions are defined as follows:

Casein. The protein fraction precipitated by acidifying raw skimmilk to pH 4.6 to 4.7. It comprises about 80% of the proteins of milk and consists of a mixture of three principal components, α-, β-, and γ-caseins *(8, 13)*. A fourth fraction was isolated from casein by Cherbuliez and coworkers *(2)* and called

δ-casein. Present information does not enable a decision as to whether δ-casein is a separate entity or a mixture of the other casein components. Casein exists in milk in complex particles containing calcium and phosphate.

Milk serum proteins (or whey proteins). The proteins of skimmilk other than casein comprising about 20% of the total milk proteins.

Heat-labile milk serum proteins. The portion of the milk serum proteins (about 80%) that is rendered acid precipitable at pH 4.6 to 4.7 by previous heat treatment of the milk (viz. at boiling for 20 minutes).

Albumin or lactalbumin fraction. The portion of the milk serum proteins that is soluble in neutral one-half saturated ammonium sulfate or saturated magnesium sulfate. In the Rowland distribution, "lactalbumin" designates the portion of the *heat labile* milk serum proteins soluble in saturated magnesium sulfate.

Globulin or lactoglobulin fraction. The portion of the milk serum proteins that is insoluble in neutral one-half saturated ammonium sulfate or saturated magnesium sulfate. In the Rowland distribution, "lactoglobulin" refers to the portion of the *heat labile* milk serum proteins insoluble in saturated magnesium sulfate.

Commercial lactalbumin. A product precipitated by heating acidified whey and including both the lactalbumin and lactoglobulin fractions.

Proteose-peptone fraction. The portion of the milk serum proteins that is not rendered acid precipitable at pH 4.6 to 4.7 by previous heat treatment of the milk.

Fat globule "membrane" proteins. A complex of proteins and enzymes including lipoproteins, alkaline phosphatase, and xanthine oxidase adsorbed on the surface of milk fat globules. This material (or at least a fraction thereof) can be isolated by washing cream and churning *(16, 19)*.

The three caseins, α, β, and γ, account for the casein fraction *(8, 12)*. α-Lactalbumin, β-lactoglobulin, and the "blood" serum albumin have been crystallized from the lactalbumin fraction and appear to account for the bulk of it *(5, 11, 18, 21)*. The eu- and pseudo-immune globulins have been isolated from the classical lactoglobulin fraction and probably account for much of it *(27)*. The "proteose-peptone" fraction appears to consist of at least three electrophoretically distinct components *(11)*, but this fraction has not been satisfactorily resolved as yet primarily because it has been prepared only from drastically heated milk and the components may represent artifacts to some extent.

In addition to the specific proteins mentioned heretofore, milk contains small amounts of other proteins and enzymes, which altogether comprise a very small percentage of the total protein. The enzymes become distributed among the various fractions on separation and in some cases are difficult to remove. For example, amylase is found in the lactoglobulin fraction, peroxidase in lactalbumin, a protease in casein, and xanthine oxidase and alkaline phosphatase in the fat globule "membrane." Two milk enzymes, peroxidase and xanthine oxidase, have been crystallized.

As individual proteins have been isolated and characterized, the classical nomenclature has become inadequate, and undoubtedly it will eventually be discarded entirely. The Committee recommends that nomenclature in terms of individual protein components be used wherever possible.

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NIPPLE VERSUS PAIL FEEDING OF MILK TO HOLSTEIN CALVES[1]

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Many conflicting claims have been made relative to the advantages or disadvantages of hand-feeding milk to calves by nipples as compared to open-pail feeding. Advocates of the nipple system feel that by forcing the calf to drink more slowly and by elevating its head the milk is directed to the abomasum, thus by-passing the rumen. Only limited research on these methods of milk feeding has been reported. Alexander *(1)* noted only slight differences in weight gains of calves fed by nipple as compared to those fed from pails for a period 24 weeks from birth. They noted a small advantage for nipple-fed calves in two trials and a rather large difference in favor of pail-fed calves in the third. Wise *et al. (5)* showed that elevation of the head had little to do with closure of the esophageal groove.

This study was conducted to determine whether the method of hand-feeding of milk had any appreciable effect on growth and health of the young calf.

EXPERIMENTAL PROCEDURE

Male Holstein calves were obtained from various Pennsylvania state institutional herds and from the University research herd. Calves were 2 to 5 days of age when brought to the experimental barn; however, the average age at which each group entered the experiment was identical. They were selected only to the extent that body weight limits were 90 to 110 lb. and that all calves appeared normal and healthy upon arrival. Upon entering the barn they were assigned at random to the following treatments: (A) Pail feeding—milk fed from metal pails placed on the floor. (B) Elevated pail—milk fed from metal pails which were elevated 10 in. above the floor. This was accomplished by placing the pail in the bunker from which grain was fed. (C) Nipple pail—this pail was suspended from the side of the stall in such a way that the calf elevated its head to approximately the same height as in natural nursing. (D) Nipple bottle—2-qt. nipple bottles, in a special metal rack, were suspended from the side of the stall at about the same height as the nipple pail.

Care and feeding of all calves was similar except for method of feeding milk. They were housed in individual, solid-walled pens in which were provided fresh water, salt, hay, and concentrate. Milk feeding followed approximately the schedule given in Pennsylvania Leaflet 146 *(3)*. In Trials 1 and 2, each calf was fed 5 lb. of Holstein herd milk twice daily until the age of 42 days. In Trial

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3, maximum milk fed was 4.8 lb. per feeding. This was done in order to avoid adding 0.2 lb. of milk to the bottle after it was emptied. All calves were fed calf starter ad libitum. This starter, which contained approximately 18% digestible protein, has given good results in previous trials at this station. Alfalfa hay of average quality was fed ad lib in all three trials. No particular measures were taken to prevent scours, other than to see that feeding utensils were kept clean and that general sanitation was practiced throughout the experiments. In cases of scours the milk allowance was reduced and, if necessary, sulfathaladine was administered orally.

Records were kept on each calf as follows: Body weight and height at withers were obtained initially and at weekly intervals thereafter, measurements being made at the same time each week. Consumption of milk, starter, and hay was noted. At weekly intervals the time required for each calf to drink was measured with a stop watch. Health records were kept, noting all cases of scours and other abnormal health conditions and the severity thereof. Data were analyzed by analysis of variance where applicable *(4)*.

The experiment was divided into three trials. In Trial 1, 16 calves were used, four on each of the above mentioned treatments. Trial 2 was conducted in the spring of 1953 and also employed 16 calves, eight each on treatments A (pail on floor) and C (nipple pail). Trial 3 was accomplished in the summer of 1954, utilizing 16 calves, eight each on treatments B (elevated pail) and D (nipple bottle). Thus, 48 calves were employed, 12 on each of the milk feeding systems. Duration of each trial was 6 weeks.

RESULTS AND DISCUSSION

Growth and feed consumption data are presented in Table 1. In Trial 1, calves fed milk from an open pail placed on the floor gained in body weight at a faster rate than those fed from elevated pails or nipple pails. Nipple bottle–fed calves gained in body weight faster than those fed from the nipple pail. These differences were significant at only the 5% level of probability. These advantages were not evident with respect to height at withers, since differences in gains at height at withers were not significant. Differences between groups with respect to starter and hay consumption were not statistically significant.

In view of the results obtained from this preliminary trial it was deemed advisable to run further trials embodying larger numbers of calves. Trial 2 utilized eight individuals on each of the two treatments in which calves were fed milk from pail on the floor and nipple pails. As shown in Table 1, there were no significant differences in growth as measured by gain in body weight and in height at withers. Starter and hay consumption by the two groups was very similar.

Trial 3 employed eight calves each on the elevated pail and nipple bottle treatments. As shown in Table 1, the calves fed from the elevated pail grew at a rate which was significantly greater than those fed from the nipple bottle. This difference amounted to an advantage of 14.6 lb. average increase in body weight and 2.1 cm. in height. Although the differences were not statistically sig-

TABLE 1

Effect of method of feeding milk to young calves to 6 weeks of age

Trial No.	Treatment	No. of calves	Growth data Increase over initial		Feed consumption per calf		Av. No. days scoured	Time required to consume milk at one feeding	
			Body wt.	Height	Starter	Hay			
			(lb.)	*(cm.)*	*(lb.)*	*(lb.)*		*(min. & sec.)*	
1	A. Pail on floor	4	45.5[a]	5.1	31.7	4.2	2.7	1	0
	B. Elevated pail	4	33.5	5.4	20.3	11.6	1.5		48
	C. Nipple pail	4	32.5	4.8	18.4	5.0	2.7	3	32
	D. Nipple bottle	4	39.7[b]	6.3	19.7	12.6	3.0	5	35
2	A. Pail on floor	8	37.1	5.8	35.7	11.1	2.4		57
	C. Nipple pail	8	38.0	6.4	35.8	11.5	2.9	3	25
3	B. Elevated pail	8	46.2[c]	4.3[c]	19.7	11.5	0.2		42
	D. Nipple bottle	8	31.6	2.2	13.5	6.8	0.0	3	28

[a] Significant at the 5% level of probability over groups B and C.
[b] Significant at the 5% level over group C.
[c] Significant at the 1% level of probability.

nificant, the data do show that the pail-fed calves ate more of both starter and hay during the 6-week period. Inspection of individual data revealed that the pail-fed calves started at an initial weight of 104 lb. as compared to 102 lb. for the nipple bottle-fed group. The pail-fed calves started growing at an accelerated rate as compared to the nipple-fed group. After 3 weeks the rate of acceleration decreased, but the calves continued to gain at a faster rate than did nipple-fed calves. The pail-fed calves ate more grain and at an earlier age, and they started to eat more hay at 3 weeks of age and continued to eat more.

Scouring was not a severe problem in these trials, although no special precautions were taken to prevent occurrence of the condition. When a calf did scour, the milk allowance was reduced for one or two feedings, and, if the condition persisted, sulfathaladine was administered. As shown in Table 1, in Trial 1 pail-fed calves scoured an average of 2.1 days per calf and nipple-fed calves scoured an average of 2.9 days. In Trial 2 the pail-fed calves scoured 2.4 days and those nipple-fed scoured 2.9 days. In Trial 3 only one calf had scours severe enough to be recorded, that calf being a member of the pail-fed group. Thus, it can be concluded that the method of feeding had little or no effect on scouring under the conditions of this study.

As expected, the pail-fed calves consumed their milk much more rapidly than did those fed via nipple. The time required by each calf to consume a full allowance of milk for one feeding was recorded at weekly intervals. A tabulation of results showed that nipple-fed calves required an average of 3 minutes, 49 seconds as compared to 52 seconds required by the pail-fed calves.

When data for combined trials were considered, the 24 pail-fed calves showed an average gain in body weight of 41.0 lb. over initial weight, the 24 nipple-fed calves, 35.2 lb. This difference was due largely to the poorer gains made by nipple-fed calves in Trial 3. Average initial starting weights were 100.5 lb. and 98.9 lb., respectively, for pail- and nipple-fed calves. Pail-fed calves ate 4.3 lb. more starter and 1.1 lb. more hay than did nipple-fed calves.

No ready explanation is apparent for the differences found in these trials. Those noted in Trial 1 could have been due to the small number of calves in each group; however, the superior growth shown by pail-fed calves in Trial 3 is not so readily explained. Individuals appeared uniform in both groups at the beginning of this later trial; however, the pail-fed calves appeared superior to nipple-fed ones after the first few weeks. It should be noted that the gains exhibited by group D calves with respect to height, and somewhat with respect to body weight, were inferior to those exhibited by any other group in the experiment. It would appear that the results of Trial 3 should be regarded in the light of poor response by nipple-fed calves rather than a superiority of the pail-feeding treatment. It can be said that nipple feeding was not superior to pail feeding in these trials.

SUMMARY

Forty-eight male Holstein calves were employed in a comparison of methods of feeding milk. Methods studied were: open pail placed on floor, open pail

elevated 10 in. off the floor, nipple pail, and nipple bottle. Calves were fed milk to 6 weeks of age. In one of three trials, pail-fed calves exhibited superior growth when compared to nipple-fed calves. This difference was not substantiated in the other two trials. Pail-fed calves consumed their milk more quickly than did those nipple-fed, and there was no difference in incidence of scours between pail- and nipple-fed calves.

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A METHOD FOR THE ISOLATION OF BOVINE LIVER ESTERASE

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In studies dealing with the isolation and characterization of the principle, tenulin, responsible for the "bitter weed flavor" in milk, it was necessary to make a comparative study between the principle in the weed as compared to that found in the milk. It had been reported by Clark *(3)* that tenulin contained an acetic acid ester linkage. Thus, it was reasoned that some attention might be paid to the action of an esterase upon this linkage. A search of the literature revealed that no satisfactory method was available for the isolation of an esterase from bovine liver *(4)*.

It was noted that horse liver esterase had been highly concentrated by a procedure reported by Connors *et al. (5)*. In their report mention is made that the procedure is not applicable for the purification of bovine liver esterase. The purification of an esterase of plant origin has been reported by Jansen *et al. (6)*. However, neither of the above reports contained information which could be used for developing a method for the purification of bovine liver esterase. In this communication a procedure is described for the purification of bovine liver esterase.

EXPERIMENTAL PROCEDURE

Bovine esterase assay. The extent of the liberation of carboxyl groups of several esters was determined by a method described by Balls *et al. (1)* for the assay of lipase activity with minor modifications. The procedure employed follows:

Into a 60-ml. glass-stoppered bottle were introduced the weighed sample of the ester and 5 ml. of bile-glycerol. The mixture was warmed in a water bath preheated to 60° C. for 10 minutes. During the heating interval the contents were shaken frequently so as to assist in forming a homogeneous mixture.

After the mixture was cooled, 10 ml. of 0.5 *M* phosphate buffer, which had been adjusted to a pH of 8.5, 0.5 ml. of saturated calcium sulfate, and two drops of 3% phenolphthalein were added. This was followed with several drops of 1 *N* ammonium hydroxide, which imparted to the mixture a distinct purplish color. After thorough mixing, 10 ml. of the enzyme preparation in 50% glycerol was added, and the final volume was adjusted to 30 ml. with water.

A constant incubation temperature of 37° C. was maintained for the assay of the different esters studied. This was realized by placing reagents, enzyme, and ester in separate 60-ml. bottles in a preheated incubator for 15 minutes prior to adding the enzyme to the final mixture. During the incubation period the assay mixture was kept uniformly mixed by means of a magnetic stirrer.

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The fatty acids liberated in the assay of triacetin, tributyrin, methyl-butyrate, and ethyl acetate were determined by titrating 5-ml. aliquots of the incubate at 30-minute intervals with 0.08 *N* alcoholic potassium hydroxide *(7)*. As the yield of fatty acid liberated for triolein during a 30-minute incubation period was small in comparison with the other esters, a solution of alkali, 0.03 *N*, was found to be more accurate for the measurement and was also used in studying tenulin.

The esters used for the above assays of bovine esterase were purified products obtained from Eastman Kodak Comapny. The tenulin material for the activity comparison was purified according to the procedure of Clark *(2)*.

The units of activity of the various enzyme preparations were determined with triacetin as substrate, a unit of activity being defined as that which hydrolyzed 0.073 g. of triacetin in 30 minutes.

Purification of enzyme. The method of preparation and purification of the enzyme together with yields follows:

Step 1. Preparation of acetone powder. A sample of 2.27 kilos of commercial beef liver was macerated with a food chopper and stirred vigorously with 2 l. of acetone. The supernatant liquid was decanted, and washing was continued with two additional aliquots of acetone and finally with portions of ethyl ether. Upon completion of the dehydration and defatting procedure, the solid material was air-dried. The recovery for this step was 13%.

Step 2. Water extraction. A 210-g. sample of the above material was pulverized in a Waring blendor and sieved through a 30-mesh screen. The powder was gently stirred with 2,100 ml. of precooled distilled water, and extraction was allowed to ensue for 12 hours with an occasional shaking at a temperature of 5° C. An aqueous extract of 1,900 ml. was obtained by centrifugation.

Step 3. Recovery of enzyme. The above extract was diluted with anhydrous acetone, ten to one, and the mixture was allowed to set at 5° C. for 3 hours. During this interval a voluminous material separated. It was collected on the filter. The gelatinous material was removed to a porous plate and air-dried. The powder recovered weighed 12.5 g. and represented on a dry weight basis a yield of 6%. This material was designated residue No. 2.

Step 4. Purification of enzyme. For further purification, Step 3 was continued with residues No. 2 and No. 3. The yield for the dry residue No. 3 was 16%.

DISCUSSION AND RESULTS

Stability of enzyme. Samples of the enzyme preparation, residues No. 2 and No. 3, were stored in brown reagent bottles at 5° C. The enzyme preparations stored under these conditions retained constant activity for at least 1 month. The enzyme solutions for assay prepared by extracting residues with 50% glycerol were never stable for periods longer than 10 days.

Activity of enzyme preparations. The activity of the various enzyme preparations, acetone powder and residues No. 2 and No. 3, was determined with triacetin by the procedure described above. An activity of three units per gram was found for the acetone powder, 30 units for residue No. 2, and 60 units for

TABLE 1
Comparative esterase activity

Bovine liver esterase		Horse liver esterase	
Acetone powder	Purified enzyme	Acetone powder	Purified enzyme
3 units per gram	96 units per gram	106 units per gram	428 units per gram

residue No. 3. When fresh liver was the starting material, residue No. 3 showed an activity of 96 units per gram.

A comparison of the units of activity per gram for bovine liver esterase and horse liver esterase is presented in Table 1. The elaborate procedure of Connors *et al. (5)* would probably account for the differences in activity.

Activity towards substrates. In comparing the activity of bovine liver esterase upon various substrates with that upon tenulin, it was found that triacetin was very readily cleaved whereas triolein was only slowly cleaved. Thus, triacetin was used to standardize the procedure used in studying the action of the enzyme upon various low molecular weight esters. The relative cleavage action of bovine liver esterase upon low molecular weight esters, and also upon tenulin, is shown in Figure 1. It is seen from the location of tenulin in Figure 1 that bovine liver esterase was without cleavage action upon this acetic acid ester.

The weight of the various esters represented in Figure 1 approximated 0.30 g. with the exception of tenulin. Samples of tenulin greater than 0.1800 g. were insoluble in an assay mixture of 30 ml., thus limiting the amount of this ester which could be studied by our procedure. The 30-ml. assay solutions contained

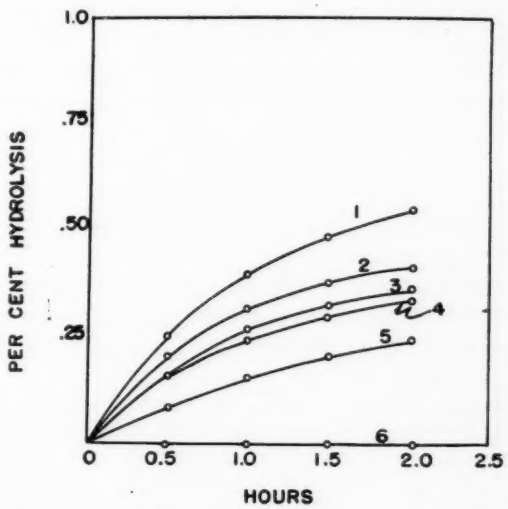

FIG. 1. The relative hydrolysis of several esters: 1, triacetin; 2, methyl butyrate; 3, ethyl acetate; 4, tributyrin; 5, triolein; 6, tenulin.

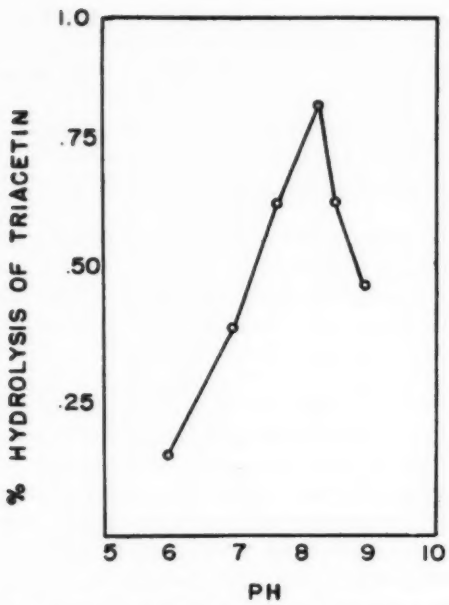

FIG. 2. The influence of pH upon bovine esterase activity.

less than one meq. (0.306 g.) of soluble material. However, tenulin was cleaved by bovine esterase at a negligible rate as compared with triacetin.

Optimum pH. The optimum pH of bovine liver esterase activity upon triacetin was determined with 0.5 *M* phosphate buffers. It is seen from Figure 2 that the optimum pH for this enzyme is approximately 8.0. This value seems to be in good agreement with the results of Connors *et al. (5)* reported for horse liver esterase.

SUMMARY

An esterase from bovine liver acetone powder has been concentrated 60-fold by water extraction and precipitation with acetone. The activity of the enzyme toward various low molecular weight substrates was studied. Triacetin was adopted as a substrate for comparative activity studies. Under the conditions of the assay, the optimum pH for esterase activity was found by using phosphate buffer to be approximately 8.0.

With reference to tenulin, an acetic acid ester, it was observed that bovine liver esterase was without action upon the ester linkage.

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EFFECT OF EGG YOLK AND ITS ISOLATED CONSTITUENTS UPON THE DEHYDROGENASE ACTIVITY OF BOVINE SPERMATOZOA[1]

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An active succinic dehydrogenase was first demonstrated in the semen of man and bull by Lardy and Phillips *(16)* and MacLeod *(22)* by use of the Thunberg technique. Subsequently, Zittle and Zitin *(41)* demonstrated the presence of cytochrome oxidase in bull spermatozoa, and Mann *(24, 25)* observed the complete cytochrome spectrum in the spermatozoa of man, ram, bull, and boar. However, MacLeod was unable to demonstrate any connection between succinate oxidation and the persistence of spermatozoan motility. Ryan *(33)* observed an increase in respiration of cold-shocked spermatozoa in the presence of succinate, which appeared to be associated with an increase in spermatozoan motility.

Although the necessity for an active succinic dehydrogenase in the maintenance of spermatozoan viability has not been established, the importance of dehydrogenases in general to spermatozoa has been confirmed by the relationships which have been observed between "reduction time" and motility, longevity, and per cent nonreturns by Beck and Salisbury *(4)* and Erb and Ehlers *(11)*.

Since the introduction of egg yolk as a desirable constituent of a semen diluent by Phillips *(29)* and Phillips and Lardy *(30)*, Tosic and Walton *(37, 38, 39)* and Lasley and Mayer *(21)* have described its effect upon spermatozoan respiration. Tosic and Walton obtained an initial stimulation of respiration followed by a decrease when egg yolk was added to ejaculated bull spermatozoa and explained the observed decrease as caused by the presence of a dialyzable organic peroxide in egg yolk. Lasley and Mayer, however, observed no respiratory effect following the addition of egg yolk to ejaculated ram spermatozoa but found an increase in the respiration of epididymal spermatozoa.

Lasley *et al. (19)*, Easley *et al. (9)*, Lasley and Bogart *(18)*, Lasley and Mayer *(20)*, and Bogart and Mayer *(6)* have suggested that egg yolk is of importance in protecting spermatozoa against adverse environmental conditions.

Fractionation of egg yolk has yielded fractions valuable in the preservation of spermatozoa. Lardy and Phillips *(17)*, Kampschmidt *et al. (14)*, and Ryan *(33)* have discussed the beneficial effects of egg yolk phospholipids and lipoproteins upon spermatozoan longevity. Kampschmidt *et al.* and Bogart and Mayer recognized two distinct egg yolk factors, one of which protects spermatozoa against the harmful effects of cold shock and a second which contributed to survival (a storage factor). Egg yolk phospholipids, particularly lecithin, have

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been identified as resistance factors by Ryan and Kampschmidt *et al.*, whereas Lardy and Phillips demonstrated the oxidative metabolism of egg yolk phospholipids whenever spermatozoa were deprived of a metabolizable hexose.

In recognition of the importance of dehydrogenases and the many beneficial effects of the addition of egg yolk to spermatozoa, as well as its effect upon spermatozoan respiration in general, a series of experiments was conducted to determine the effect of egg yolk in the medium upon the dehydrogenases concerned with carbohydrate metabolism of spermatozoa.

MATERIALS AND METHODS

The semen was collected from healthy dairy bulls maintained as a regular part of the University of Missouri Experiment Station dairy herd. An artificial vagina was used to collect the semen, which was immediately placed in a Thermos bottle of water at a temperature no lower than 15° C. for transport to the laboratory. When received at the laboratory, the vial of semen was placed in a 400-ml. beaker of water and stored in the refrigerator. The large water volume insured gradual cooling to a storage temperature of 4° C., thereby preventing cold shock.

Generally, the semen specimens from two or more bulls were pooled. If the spermatozoa were to be washed, they were mixed with about five volumes of 0.9% saline. The wash solution and spermatozoa were separated by centrifugation at 950 G or 2,200 r.p.m. in a Servall model G centrifuge for about 10 minutes. The seminal plasma saline mixture was removed by use of a hand controlled automatic pipette. The washing process was repeated four times, after which the spermatozoa were resuspended in sufficient isotonic saline to attain the original semen volume.

Spermatozoan concentration counts were made by using an eosin counting fluid as described by Smith and Mayer *(35)*. Five counts were made on each sample, and their average was used to calculate the cubic millimeters of CO_2 liberated by 10^8 spermatozoa per hour.

The Warburg flasks were cleaned by the potassium permanganate–sodium hydroxide method and calibrated by fillling them with mercury, according to the method of Umbreit *et al. (40)*.

Since spermatozoan suspensions are difficult to prepare with sufficient optical transparency to facilitate application of spectrophotometric techniques for the determination of enzyme activity, the manometric method of Quastel and Wheatley *(31)* was employed to estimate the dehydrogenase activity of the spermatozoa in the different suspending media. This method, used more recently by Rubinstein and Denstedt *(32)* in an investigation of the metabolism of the avian erythrocyte, uses ferricyanide as an artificial hydrogen carrier to liberate CO_2 from a bicarbonate medium. The ferricyanide was placed in a side arm of the Warburg flasks and tipped in after the equilibration period at zero time to initiate the reaction, and the manometric readings were recorded at 5-minute intervals for a period of 1 hour.

Egg yolk was added to the flasks as a 1:1 mixture of egg yolk and 1.3% $NaHCO_3$. The volume of the mixture added to each flask was 0.6 ml., equivalent

to 0.3 ml. of egg yolk per flask. Egg yolk lecithin was prepared by the method of Pangborn *(28)*. Twelve mg. of lecithin prepared in this manner was added to each flask as an emulsion.

The cholesterol, a commercial preparation,[3] was incorporated into a water emulsion by dissolving 275 mg. in 15 ml. of hot methanol and adding the hot solution to 10 ml. of water. A volume of 0.6 ml. of this emulsion, containing 6.6 mg. of cholesterol, was added to each reaction flask.

The β-carotene emulsion was prepared by mixing 3 ml. of a synthetic β-carotene solution in petroleum ether (50×10^{-6} g. per milliliter) with 3 ml. of mineral oil and 3 ml. of d.d. H_2O and the petroleum ether, removed in vacuo. Then 1 ml. of the lecithin preparation in absolute alcohol, in a concentration of 300 mg. of lecithin per milliliter, was added as an emulsifying agent. Exactly 0.3 ml. of this emulsion was added to each flask to give 4×10^{-6} g. of carotene per flask, or about the same amount as is contained in 0.3 ml. of egg yolk. To rule out the effect of either lecithin or mineral oil, 0.3 ml. of a solution containing the above ingredients with the exception of carotene was also added to the control flasks.

A crude flavine-adenine-dinucleotide concentrate was prepared by the method of Dimant *et al. (8)*, with the exception that egg yolks were used instead of pig liver or yeast. The egg yolks were fractionated into acetone soluble and insoluble fractions by the method of Pangborn *(28)*.

Skimmilk, obtained from the University of Missouri Dairy Department, was used unheated in some experiments and for others was heated in a water bath at 92° C. for 10 minutes.

RESULTS

The data showing the effect of whole egg yolk upon the dehydrogenase activity are presented in Table 1. These data demonstrate an apparent stimulation by egg yolk of all of the dehydrogenases studied in this investigation. Further, they show a highly significant stimulation of the succinic dehydrogenase activity of both washed and unwashed bovine spermatozoa following addition of the egg yolk mixture. The effect of egg yolk upon the succinic dehydrogenase activity of washed and unwashed spermatozoa is approximately the same, an increase of about 25 cu. mm. CO_2. Since fewer samples of the unwashed spermatozoa were available for study, the results appear to be less significant. Although the data (Table 1) indicate a slight stimulatory effect of egg yolk upon glyceraldehyde-3-phosphate and malic dehydrogenases, these data were not statistically significant.

The value of lecithin to spermatozoan viability has been established by Kampschmidt *et al. (14)*, Lardy and Phillips *(17)*, and Ryan *(33)*, and its importance to the succinic oxidase system in homogenized muscle cell preparations was reported by Nygaard and Sumner *(27)* and Edwards and Ball *(10)*. Hence, it seemed logical that lecithin might be responsible for the dehydrogenase stimula-

[3] Cholesterol preparation obtained from the American Lecithin Co.

TABLE 1

Effect of egg yolk upon bovine spermatozoan dehydrogenase activity

Dehydrogenase system	Cu. mm. CO_2 liberated per 10^8 spermatozoa per hour	
	No egg yolk added	Added 0.6 ml. of 1:1 egg yolk:1.3% $NaHCO_3$
Succinic a[a]	32.0 (8)[c]	57.7
Succinic b[b]	30.6 (4)	55.6
Glyceraldehyde-3-phosphate	6.8 (9)	8.6
Malic	10.8 (3)	11.1

[a] $P = < 0.01$.
[b] $P = < 0.05$.
[c] Values in parentheses indicate number of runs which were averaged.

Conditions: all spermatozoa except those used in succinate b, which were unwashed, were washed four times with isotonic saline and resuspended in sufficient saline to reattain the original seminal volume.

Flask contents: for all systems, $NaHCO_3$, 1.86×10^{-2} *M*, $K_3Fe(CN)_6$ 0.2 ml. of an 11% solution neutralized with $NaHCO_3$, 0.3 and 0.5 ml. of spermatozoan suspension, sufficient saline to make the total flask volume 2.5 ml., added when measuring (succinic dehydrogenase) Na-succinate, 0.08 *M*, glyceraldehyde-3-phosphate dehydrogenase, DPN 3×10^{-4} *M*, nicotinamide 0.03 *M*, malic dehydrogenase, DPN 3×10^{-4} *M*, nicotinamide 0.3 *M*, Na-l-malate 0.8 *M*, NaCN 0.2 ml. of a solution of 0.65 *M* neutralized with HCl.

Gas phase: 5% CO_2 95% N_2, incubation temperature 38° C., time 1 hour; values are expressed as cu. mm. CO_2 liberated per 10^8 spermatozoa per hour, after correction for a blank containing no substrate.

tion effect of egg yolk. However, the results (Table 2) show a slight inhibition of succinic dehydrogenase activity in the presence of lecithin.

The failure to find a stimulation of succinic dehydrogenase in the presence of lecithin suggested that another egg yolk constituent was enhancing the enzyme activity. Succinic dehydrogenase has been shown to be dependent upon riboflavin and flavine-adenine-dinucleotides for maximal activity by Axelrod *et al. (2)* and Ball and Cooper *(3)*. Therefore, an egg yolk concentrate of flavine was tested for its effect upon succinic dehydrogenase activity without success, as shown by the limited data presented in Table 2.

Because of an inability to associate these two egg yolk constituents, known to affect succinic oxidase systems, with spermatozoan succinic dehydrogenase stimulation, isolation of some egg yolk fractions with organic solvents was performed in an attempt to associate the active substance with a particular fraction

TABLE 2

Effect of egg yolk fractions and milk upon the succinic dehydrogenase activity of bovine spermatozoa

Factor added	Cu. mm. CO_2 liberated per 10^8 spermatozoa per hour	
	No addition	Factor added
12 mg. lecithin	37.1 (6)[a]	20.9
0.6 ml. FAD concentrate	45.5 (2)	37.2
0.6 ml. acetone soluble	45.8 (4)	64.7
0.6 ml. acetone insoluble	42.8 (6)	48.2
0.6 ml. heated milk	50.9 (2)	54.1
0.6 ml. unheated milk	50.9 (2)	47.4

[a] Values in parentheses indicate number of samples averaged.

Conditions are the same as given for succinic dehydrogenase estimation in Table 1 except that all spermatozoa were washed.

of egg yolk. The data (Table 2) suggest that the active substance was present in the acetone soluble fraction of egg yolk.

The importance of cholesterol to cellular metabolism, its effect on the diffusion of acids and alkalis into gelatin and agar, and its beneficial effect of preventing hemolysis by mumps virus hemolysin have been reported by Affonsky *(1)*, Magistris *(23)*, and Morimoto and Morgan *(26)*. Since cholesterol comprised a large percentage of the acetone soluble fraction of egg yolk and possessed the properties cited, its effect on bovine spermatozoan dehydrogenases was measured. A highly significant activation of succinic dehydrogenase by cholesterol ($P = < 0.01$) was observed (Table 3). However, a significant inhibition of glyceraldehyde-3-phosphate dehydrogenase and an apparent inhibition of malic dehydrogenase were observed (Table 3).

TABLE 3

Effect of cholesterol upon bovine spermatozoan dehydrogenase activity

Dehydrogenase system	Cu. mm. CO_2 liberated per 10^8 spermatozoa per hour	
	No cholesterol added	6.6 mg. cholesterol added to each flask
Succinic [a]	49.5 (15)	61.9
Glyceraldehyde-3-phosphate [b]	6.3 (10)	3.6
Malic	10.3 (4)	8.1

[a] $P = 0.01$.
[b] $P = 0.05$.
Conditions are the same as in Table 1 except that all spermatozoa were washed.

Since the entire stimulating effect of egg yolk could not be attributed to cholesterol, one or more additional egg yolk constituents were possibly involved. Herisset *(12)* found that, in the presence of methylene blue as a hydrogen acceptor, the addition of a small amount of carotene-in-oil emulsion accelerated the action of Schardinger enzyme, succinic dehydrogenase, and glutamic acid dehydrogenase. On the basis of these results, the effect of carotene emulsions upon malic and succinic dehydrogenases of bovine spermatozoa was investigated. The effect of carotene, another component of the acetone soluble fraction of egg yolk, upon bovine spermatozoan succinic and malic dehydrogenases is presented in Table 4. These data show that carotene stimulates the activity of both succinic and malic dehydrogenases.

In order to determine if heated milk, which has been shown to be a satisfactory semen extender, would also activate succinic dehydrogenase, a comparative

TABLE 4

Effect of carotene upon the succinic and malic dehydrogenase activity of bovine spermatozoa

Dehydrogenase system	Cu. mm. CO_2 liberated per 10^8 spermatozoa per hour	
	No carotene added	4×10^{-6} g. carotene added per flask
Succinic	25.8 (6)	34.6
Malic	10.0 (3)	20.1

Conditions are the same as in Table 1 except that all spermatozoa were washed.

determination with unheated milk was made. It was found (Table 2) that heated milk has a slight apparent stimulatory effect upon succinic dehydrogenase, whereas unheated milk, which is spermicidal, was slightly inhibitory in its effect upon bovine spermatozoan succinic dehydrogenases.

DISCUSSION

Activation of the succinic and malic dehydrogenase systems of bovine spermatozoa by egg yolk or certain of its constituents appears to be compatible with previous evidence obtained in this and the Wisconsin laboratories. Smith *et al. (34)* demonstrated that, in an egg yolk diluent, maintenance of an osmotic pressure within certain optimal limits was dependent upon the addition of a metabolyzable hexose. Unpublished observations in this laboratory indicate that the buffering capacity of the egg yolk-glucose-$NaHCO_3$ diluent *(13)*, though low, is sufficient to prevent a dangerous shift towards an acid pH throughout a 10-day storage period. Lardy and Phillips *(17)* obtained evidence suggesting that spermatozoa metabolized egg yolk phospholipids in the absence of a metabolizable hexose. It is possible, then, that the egg yolk activated dehydrogenases function to remove the acid products of glycolysis in a diluent, thereby maintaining both a physiological pH and osmotic pressure.

The apparent failure of egg yolk lecithin to stimulate the succinic dehydrogenase activity of bovine spermatozoa is interesting in view of the excellent results obtained with lecithin in protecting spermatozoa against adverse environmental conditions. However, it may be recalled that two egg yolk factors, one a storage and the other a resistance factor, were recognized in egg yolk and that lecithin was identified as a resistance factor. Could it be that lecithin and/or lecitho-protein complexes act as resistance factors whereas cholesterol and/or carotene, or complexes of these compounds, may be classified as storage factors?

Cholesterol, on the other hand, has not been closely associated with cellular metabolic activities by investigations in this field. The literature has cited the effects of cholesterol upon membrane permeability and the marked ability of this hydrophobic compound to form water-in-oil emulsions, thus holding water in the tissues. Cholesterol, also, counteracts the effects of Ca^{++} upon lecithin when the latter is in contact with water and tends to reduce the surface occupied by the lecithin, according to Bloor *(5)*. The only apparent explanation of the stimulation of succinic dehydrogenase activity by cholesterol would seem to be its effect upon cellular permeability. This effect may be a direct one or it may be an indirect effect in which it prevents Ca^{++} inhibition of lecithin. The indirect effect is valid if it be assumed, as it was for the erythrocyte by Morimoto and Morgan *(26)*, that cholesterol and lecithin are components of the spermatozoan cellular membrane. Investigation of the chemistry of mammalian spermatozoa now in progress in this laboratory suggests the validity of this assumption.

The results also show that cholesterol inhibits glyceraldehyde-3-phosphate and malic dehydrogenase activity of bovine spermatozoa. This finding suggests another possible explanation of the mechanism whereby cholesterol stimulates succinic dehydrogenase activity. The two enzyme systems, which are inhibited

by cholesterol, have at least one common property that may influence succinic dehydrogenase activity—they both require the participation of DPN for activity. DPN has been shown by Keilin and Hartree *(15)* to inhibit succinic dehydrogenase activity of muscle preparations and by Smith and Mayer *(36)* to inhibit succinic dehydrogenase activity of bovine spermatozoa. This inhibition by DPN is effected by the catalysis of the reaction malate → oxalacetate, oxalacetate being inhibitory in concentrations as low as $10^{-4}M$. Therefore, it may be that the stimulatory effect of cholesterol upon bovine spermatozoan succinic dehydrogenase and its inhibitory effect upon glyceraldehyde-3-phosphate dehydrogenase may be the result of its inhibition in some manner of DPN catalysis. Some reservation is placed upon this explanation, however, since the stimulation of succinic dehydrogenase by cholesterol occurred in washed spermatozoa, which have been shown by Smith and Mayer to be deficient in DPN. However, since it is the DPN catalysis of malate → oxalacetate which is inhibitory, any inhibition of malic dehydrogenase would result in an apparent succinic dehydrogenase stimulation.

Explanation of the mechanism of stimulation of bovine spermatozoan dehydrogenase activity by carotene stimulation is difficult. The only known function of carotene in enzyme systems is its postulated role in photosynthesis by Davis *(7)*, who assumes that carotene functions in photosynthesis as an electron transport system by virtue of its large number of resonating double bonds. It does not seem unreasonable that it might function in the bovine spermatozoan succinic dehydrogenase system in a similar manner, but there is no affirmative evidence.

SUMMARY

The effect of whole egg yolk and its constituents upon the dehydrogenase activity of bovine spermatozoa was determined. Whole egg yolk was found to stimulate the succinic, malic, and glyceraldehyde-3-phosphate dehydrogenase activity of these cells. The dehydrogenase-stimulating effect of egg yolk was found to be associated with the acetone-soluble fraction of the yolk and appeared to be the result of the stimulatory effect of cholesterol and of carotene upon succinic dehydrogenase activity. Cholesterol was observed to inhibit both glyceraldehyde-3-phosphate and malic dehydrogenase activity of bovine spermatozoa.

The importance and possible mechanisms of dehydrogenase stimulation and inhibition by egg yolk, cholesterol, and carotene have been discussed, as well as the apparent failure of lecithin to stimulate succinic dehydrogenase activity.

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A PHOSPHATASE TEST FOR DETERMINING HEAT TREATMENT OF ALFALFA MEAL

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Alfalfa meal of highest nutritional value for use in feeds can be prepared only from hay that has been heat-dehydrated promptly after cutting. Alfalfa is dehydrated artificially in a current of hot air to preserve its quality and nutritional value and especially to reduce the loss of carotene, much of which is destroyed by exposure to sun and weather in field curing. Carotene, precursor of vitamin A, is a very valuable nutritional constituent of livestock feeds. Dehydrated alfalfa meal, used as a component of feeds for poultry and animals, is an important and practical source of carotene.

Shepherd *et al. (8)* pointed out that dehydration, entailing a shorter period of exposure in the field and a shorter time from cutting to storage than other harvesting methods, results in smaller losses of leaves, digestible nutrients, green color, and carotene. Certain investigations including analyses *(2, 3, 4, 8)* have shown that artificially dried and ground alfalfa may contain between six and 21 times as much carotene as field-cured alfalfa. Moreover, the stability of carotene in alfalfa is increased by heat treatment *(5, 9)*, and this increased stability is attributed largely to heat inactivation of the carotene-destroying enzyme system *(9)*, referred to also as carotene oxidase *(5)*.

Inferior and less expensive meal can be prepared at any time of the year by grinding field-cured hay. It is easily possible for feed manufacturers to adulterate the heat-dehydrated product by adding field-cured, ground hay to the meal. A sensitive, reliable test is needed, therefore, for detecting such adulteration.

Experiments were conducted to develop a phosphatase test for this purpose. The resulting test is similar to tests employed for detecting underpasteurization of dairy products. Like milk, alfalfa contains an alkaline phosphatase enzyme that is inactivated by heat. The temperatures employed in artificial dehydration of alfalfa—commonly 250° to 350° F. at the outlet—are far in excess of those employed in pasteurization and therefore are sufficient to inactivate the enzyme completely. As the enzyme remains active in field-cured hay, its presence in what is offered as heat-dehydrated meal indicates adulteration with field-cured hay or other nondehydrated material, or too low a temperature of dehydration.

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METHODS

Principle. Essentially, the method is based on the fact that alfalfa contains an enzyme, phosphatase, which is inactivated by heat. A sample of alfalfa is treated with disodium phenyl phosphate in a buffer at the proper pH. If the enzyme is present, phenol is liberated. Interfering proteins are removed by precipitation with trichloroacetic acid and filtration. The phenol-containing solution is then made alkaline and treated with "BQC," forming blue indophenol, whose concentration is a measure of the phosphatase present.

Reagents.

1. *Buffers:*

a. *Buffer for washing meal.* Dissolve 3.18 g. of anhydrous sodium carbonate (Na_2CO_3) and 5.88 g. of anhydrous sodium bicarbonate ($NaHCO_3$) in distilled water and dilute to 1 l. (pH 9.6).

b. *Incubation buffer.* Dissolve 8.0 g. of sodium carbonate and 4.0 g. of sodium bicarbonate in distilled water and dilute to 1 l. (pH 10.1).

c. *Color development buffer.* Dissolve 17.5 g. of sodium metaborate ($NaBO_2$) and 10.0 g. of sodium chloride (NaCl) in water and dilute to 1 l. (pH 10.2).

2. *Buffer substrate:*

Add 0.1 g. of phenol-free, crystalline disodium phenyl phosphate[1] (substrate) to 100 ml. of incubation buffer. If substrate contains free phenol, first extract phenol from it as described earlier *(1, 6)*.

3. *Precipitant:*

Dissolve 42.0 g. of trichloroacetic acid (CCl_3COOH) and 0.05 g. of copper sulfate ($CuSO_4 \cdot 5H_2O$) in water and dilute to 100 ml.

4. *BQC (2,6-dibromoquinonechloroimide):*

Dissolve 50 mg. of BQC[1] powder in 10 ml. of absolute ethyl or methyl alcohol and transfer to a brown dropper bottle. This reagent remains stable for at least a month if kept in the ice tray of a refrigerator. Do not use it after it begins to turn brown.

Preparation of test samples. Before testing, the samples must first be washed free of chlorophyll and other colored, interfering substances. The following preparation and washing procedure is recommended. If hay is to be tested, grind it thoroughly. A Wiley mill is suitable for grinding. Weigh 1.5 g. of sample (meal or ground hay) and transfer it to a 150-ml. beaker. Add 50 ml. of washing buffer (resulting pH about 9.3), stir well, and allow to soak 15 minutes with occasional stirring. Filter through a Büchner funnel with suction, using Reeve-Angel No. 711 filter paper or paper of equivalent characteristics. Wash five times with 50 ml. of water each time. Dry overnight at room temperature. Adequate dryness is indicated by meal not sticking to spatula when stirred.

[1] Obtainable relatively pure, from Applied Research Institute, 2 East 23rd St., New York 10, N. Y. Mention of this firm implies no especial endorsement by the Department of the quality of its product; nor is it recommended in preference to other firms not mentioned that may produce the same product.

Preparation of controls. The control samples must be heated to destroy phosphatase. Weigh 5 g. of unwashed meal, obtained from the same sample that is to be tested, into a 250-ml. beaker. Add 100 ml. of water and boil almost to dryness. Place in oven at 212° F. and leave overnight. Then weigh 1.5 g. and wash as described above.

Conducting the test. Weigh 0.25 g. of washed meal, prepared as described under *Preparation of test samples,* into a test tube 16 or 18 × 150 mm. Weigh 0.25 g. of heated, washed meal, prepared as described under *Preparation of controls,* into another test tube. From this point, treat tests and controls alike. Add 10.0 ml. of buffer substrate to each. Mix thoroughly and incubate in water bath at 99°-100° F. (37°-38° C.) for 60 minutes. Add 1.0 ml. of precipitant, slowly, and shake carefully. Cover the tube lightly and invert several times. Permit gas to escape occasionally to prevent excessive pressure and spattering. Filter contents through 9-cm. No. 42 Whatman or filter paper of equivalent characteristics. Transfer 5.0 ml. of filtrate to another tube (preferably graduated at 5.0 and 10.0 ml.) and add 5.0 ml. of color development buffer (pH of mixture 9.3-9.4). Add 2 drops of BQC solution and mix thoroughly. Appearance of blue color indicates the presence of material that has not been dehydrated with heat. Allow color to develop for 30 minutes at room temperature or 15 minutes at 99°-100° F. Evaluate intensity of blue color preferably photometrically, employing a 610-mμ filter, or visually by comparison with standards prepared with known quantities of phenol. Subtract the value of the control from that of the test. Details of methods for preparing standards and measuring color intensities are available in descriptions of the Sanders-Sager *(1, 6, 7)* phosphatase test.

Calculation of results: γ (micrograms or units) of phenol $\times \frac{11}{5} = \gamma$ of phenol per 0.25 g. of sample.

EXPERIMENTAL PROCEDURE

Experiments were conducted to determine the range of pH at which the phosphatase exhibited its activity. A series of phosphatase tests was made by using the foregoing procedure, except that just prior to incubation, varying increments of hydrochloric acid or sodium hydroxide were added to yield incubation pH values over a range of 4.6 to 10.8. The results in Figure 1 (optimal pH curve) show that alfalfa contains both acid and alkaline phosphatase, the pH optima being approximately 5.0 and 10.0, respectively, in test samples incubated for 1 hour. The phosphatase activity in unheated meal was generally about ten times as great at pH 5 as at pH 10. However, the acid enzyme was found to be more resistant to heat, only about 90% being inactivated by heating at the usual dehydration temperatures. The alkaline phosphatase was completely destroyed by heat dehydration, and therefore a test for alkaline phosphatase was employed.

Tests were conducted to determine phosphatase values of samples containing different proportions of field-cured hay meal in heat-dehydrated meal, to indicate results of different proportions of adulteration. Figure 2 shows the average of

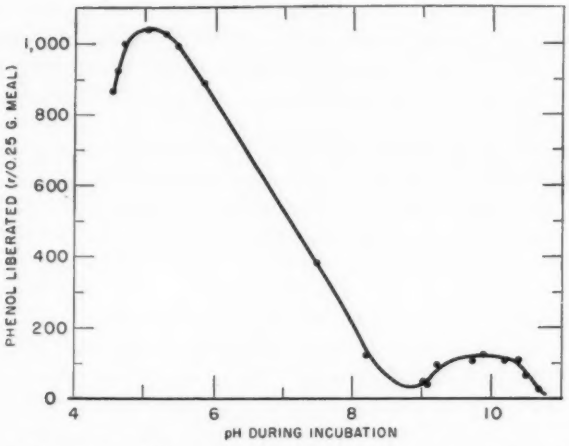

FIG. 1. Phenol liberated at different pH values in the phosphatase test on alfalfa meal prepared from field-cured (nonheat-dehydrated) hay, 1-hour incubation.

four phosphatase tests with each of eight different percentages of field-cured meal in heat-dehydrated meal. The blue color yielded by as little as 1 γ of phenol in the test, or approximately 2 γ of phenol per 0.25 g. of meal used, can be seen visually and readily detected. The data show the test to be sufficiently sensitive to detect as little as 1% adulteration with field-cured meal (phosphatase positive) added to properly heat-dehydrated meal (phosphatase negative). The test was only roughly quantitative with respect to the proportions between quantities of phenol liberated and percentages of adulteration, and a strictly linear (straight-line) relationship was not obtained (Figure 2).

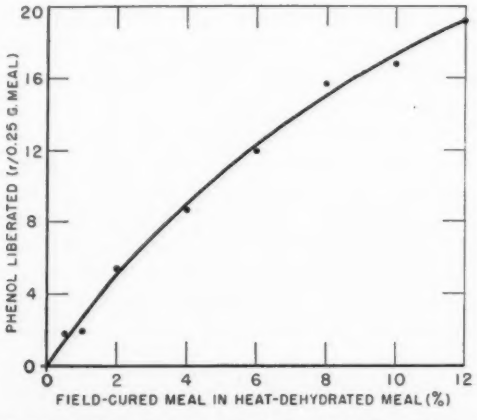

FIG. 2. Results of phosphatase tests on samples containing various percentages of field-cured alfalfa meal in heat-dehydrated alfalfa meal.

A phosphatase test for dairy products was published earlier by Sanders and Sager *(7)* and later was adopted as an official method *(1)* for determining whether or not milk and other dairy products have been adequately pasteurized. The methodology is very similar to that of the test described above, the principal differences being in the buffers and the precipitant.

In developing a practical and effective test for alfalfa meal, it was of interest to compare results obtained by the test described herein with those obtained by the Sanders-Sager (S-S) test. All the details of the S-S procedure applied to alfalfa meal were the same as the S-S procedure applied to milk, except as follows: A 0.25-g. sample of meal was used, the test samples were not heated after incubation, the protein precipitant contained 5.0 g. of zinc sulfate and 0.5 g. of copper sulfate diluted to 100 ml. in water, and the color development buffer contained 8.0 g. of sodium metaborate and 20.0 g. of sodium chloride per liter.

Table 1 shows comparative data obtained with the S-S procedure and with the Sanders-Hupfer (S-H) procedure as described above. The S-S test yielded lower control values (less off color) than did the S-H test. However, the S-H test was somewhat more sensitive in detecting the presence of field-cured alfalfa meal, and accordingly it is recommended in preference to the S-S test for this purpose.

DISCUSSION

Chlorophyll and other compounds in alfalfa interfere in the test and must be removed before testing. Dilute alkaline solutions are effective for this purpose. A sodium carbonate-bicarbonate washing buffer, described above under ***Reagents,*** was selected because it effectively removes interfering chromophoric compounds and its pH is not so high as to cause appreciable inactivation of the enzyme.

The alkaline phosphatase in alfalfa is considerably more resistant to heat inactivation than the phosphatase in milk. Its heat resistance varies somewhat in different varieties and samples of alfalfa. With some varieties, heating a 0.25-g. sample in 3.0 ml. of incubation buffer to 203° F. (95° C.) is sufficient for complete inactivation. The alkaline enzyme in all varieties and samples tested was completely inactivated by prolonged heating in water at 212° F., described above under *Preparation of controls.*

This method may not detect the presence of field-cured hay that has been subsequently heated.

SUMMARY

Alfalfa meal of the best quality for use in feeds must be prepared by artificial heat dehydration to minimize destruction of carotene or pro-vitamin A and to reduce deterioration of other nutritive qualities. A modified phosphatase test was developed to fill the need for a reliable, sensitive method for detecting adulteration of heat-dehydrated alfalfa with nutritionally inferior and less expensive field-cured alfalfa and other nonheat-treated materials.

Alfalfa hay contains both acid phosphatase and alkaline phosphatase. Only the alkaline enzyme is completely inactivated by usual temperatures employed

TABLE 1

Results of phosphatase tests on alfalfa hay meal

Sample No.	Sanders-Sager procedure[a]				Sanders-Hupfer procedure[b]			
	Photometric reading[c]			Phenol equivalent	Photometric reading			Phenol equivalent
	Sample	Control (heated)	Difference		Sample	Control (heated)	Difference	
				(γ/0.25 g.)				*(γ/0.25 g.)*
				Heat-dehydrated meal				
H-D 1	72	70	2	0[d]	72	67	5	0
	65	64	1	0	73	68	5	0
2	88	83	5	0	77	81	−4	0
3	78	78	0	0	85	90	−5	0
Av.	76	74	2	0	77	77	0	0
				Field-cured hay meal				
F-C 1	335	70	265	49	552	77	475	87
	295	70	225	41	470	80	390	71
2	565	65	500	92	578	66	512	94
	491	71	420	77	631	66	565	104
3	1780	80	1700	312	1483	98	1385	254
4	236	81	155	28	319	99	220	40
5	860	70	790	145	761	76	685	126
6	944	99	845	155	838	103	735	135
7	—	—	—	—	872	62	810	148
8	729	49	680	125	868	68	800	147
9	830	60	770	141	1094	64	1030	189
	816	51	765	140	1082	62	1020	187
10	777	77	700	128	1011	71	940	172
	886	61	825	151	932	62	870	159
11	802	62	740	136	958	73	885	162
	1036	56	980	180	841	71	770	141
Av.	759	68	691	127	831	75	756	139

[a] Barium borate-hydroxide incubation buffer.
[b] Sodium carbonate-bicarbonate incubation buffer.
[c] Klett-Summerson photoelectric colorimeter used, with 610 mμ filter.
[d] Differences of 6 scale divisions (0.5 γ) or less recorded as 0 γ phenol.

in artificial heat dehydration, and therefore a test for alkaline phosphatase activity was developed.

The test was found to be reliable and roughly quantitative with respect to the proportion of nondehydrated meal in a mixture. As little as 1% adulteration with nondehydrated material in high-quality, heat-dehydrated meal was detected regularly. The test therefore offers purchasers of alfalfa meal a valuable indication of nutritional quality.

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A METHOD FOR THE ESTIMATION OF PEROXIDASE ACTIVITY IN MILK[1]

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Most of the methods for the estimation of peroxidase activity have been colorimetric and have utilized a variety of donors, such as pyrogallol *(2, 7, 8, 9)*, guaicol *(3, 4)*, and *p*-phenylenediamine *(5, 6)*. Some methods require considerable time; some require a great deal of equipment; others require involved extraction techniques. As a result, a study was made to develop a method for determining peroxidase activity in milk having the technical advantages of speed, convenience, and sensitivity.

EXPERIMENTAL PROCEDURE

Materials. Purified lactoperoxidase was prepared by the method of Elliott *(1)*. The enzyme preparation was lyophilized and stored in a desiccator until used.

A 0.3 *N* solution of hydrogen peroxide was prepared daily by diluting 1.75 ml. of 30% hydrogen peroxide to 100 ml. with cold distilled water. The solution was standardized with potassium permanganate and kept in an ice bath when not in use.

A 2.0% solution of *p*-phenylenediamine was prepared by dissolving 1.0 g. of *p*-phenylenediamine in hot distilled water, filtering the solution while hot, and then making it up to a volume of 50 ml. when cooled.

Procedure. After tempering all materials to 20° C., 0.1 ml. of milk and 0.5 ml. of *p*-phenylenediamine were added to a colorimeter tube containing 40.0 ml. of distilled water. The contents of the tube were mixed, 0.1 ml. of hydrogen peroxide was then added, and the contents again were mixed. Five readings at intervals of 30 seconds were made on a Lumetron colorimeter equipped with a 490 mμ filter.

To determine nonenzymatic oxidation, a run was made on each sample of milk, by using the same procedure as outlined above except that hydrogen peroxide was omitted.

All samples were read against a *p*-phenylenediamine blank, prepared by mixing 0.1 ml. hydrogen peroxide and 0.5 ml. *p*-phenylenediamine with 40.1 ml. of distilled water. Since slow nonenzymatic oxidation of *p*-phenylenediamine occurred, fresh blanks were prepared every 10 minutes.

Standard curve. Peroxidase activity was compared to a standard curve of oxidized *p*-phenylenediamine. Twenty mg. of *p*-phenylenediamine were completely oxidized by 1 ml. of 2.0 % copper sulfate and 1 ml. of 0.3 *N* hydrogen

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peroxide in a total volume of 200 ml. Various amounts of the *p*-phenylenediamine were added to the nonoxidized *p*-phenylenediamine such that the total amount of both oxidized and nonoxidized *p*-phenylenediamine was constant. The optical densities of these solutions were read at 490 mμ in the Lumetron colorimeter.

Comparative method. The Sumner and Gjessing (7) method for the determination of peroxidase activity was used for comparative purposes. In general this method depends upon the extraction by ether of purpurogallin formed from pyrogallol and measurement of the optical density of the extract at 440 mμ.

RESULTS AND DISCUSSION

Studies of the kinetics of purified lactoperoxidase showed that the reaction was a first order reaction for the first 3 minutes (Figure 1). An analysis of variance of the data indicated that the line (log a/a-x vs. time) was linear and that curvature was not significant. Inasmuch as the kinetics of the lactoperoxidase system follow a first order reaction, it is most convenient to express the change in optical density by oxidized *p*-phenylenediamine as a logarithmic function of concentration.

A standard curve of optical density plotted against the logarithm of the ratio of the total concentration of *p*-phenylenediamine (both oxidized and nonoxidized)

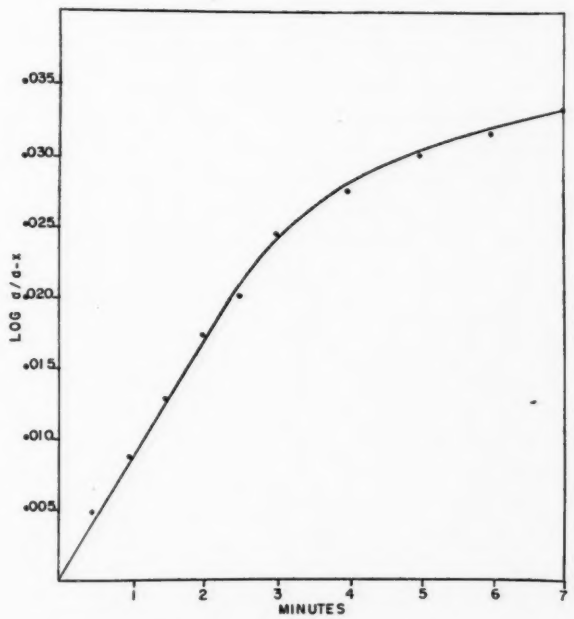

FIG. 1. Activity-time curve.

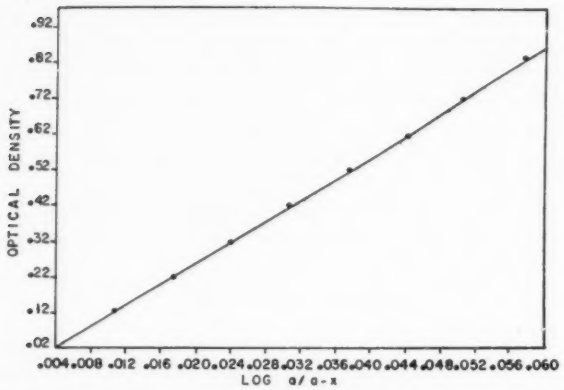

FIG. 2. A standard curve of optical density plotted against Log a/a-x. a = initial concentration of *p*-phenylenediamine; x = decrease after the lapse of time T (amount of oxidized *p*-phenylenediamine); a-x = amount of *p*-phenylenediamine remaining in the given volume.

to the concentration of nonoxidized *p*-phenylenediamine is given in Figure 2. The concentration of the total amount of *p*-phenylenediamine is the same as that used in the proposed assay procedure.

Studies showed that 0.5 ml. of 2.0% *p*-phenylenediamine was satisfactory for the measurement of peroxidase activity in milk.

Willstaetter and Stoll *(8)* have shown that hydrogen peroxide is inhibitory toward lactoperoxidase. Therefore, varying concentrations of hydrogen peroxide were used to determine the optimum peroxide concentration. It will be seen from the results presented in Figure 3 that 0.015 *M* hydrogen peroxide is the optimum concentration.

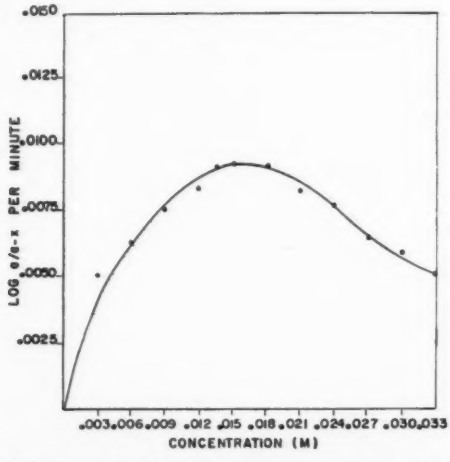

FIG. 3. The effect of hydrogen peroxide concentration upon the activity of lactoperoxidase.

In view of the fact that hydrogen peroxide is inhibitory toward lactoperoxidase, a single hydrogen peroxide concentration may not serve to compare the lactoperoxidase activity of different milk samples. It was desirable, therefore, to establish a range over which the *p*-phenylenediamine method could be used. Accordingly, a milk having high lactoperoxidase activity (1.3 units/0.1 ml.) was selected for establishing such a range. A portion of this milk was heated to 80° C. for 10 minutes to destroy lactoperoxidase activity. Varying ratios (v/v) of the heated milk to the unheated milk were used to determine lactoperoxidase activity. The results presented in Figure 4 show that the proposed method will give good results for milks having a lactoperoxidase activity over the range of 0.2-1.3 units/0.1 ml. sample. Aliquot adjustments must be made for milk samples having lactoperoxidase activity outside of the range indicated above.

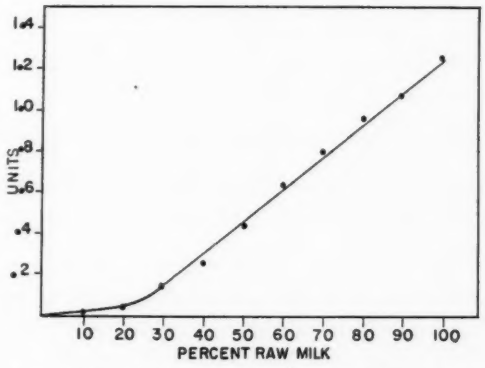

FIG. 4. Activity—lactoperoxidase concentration curve for milk.

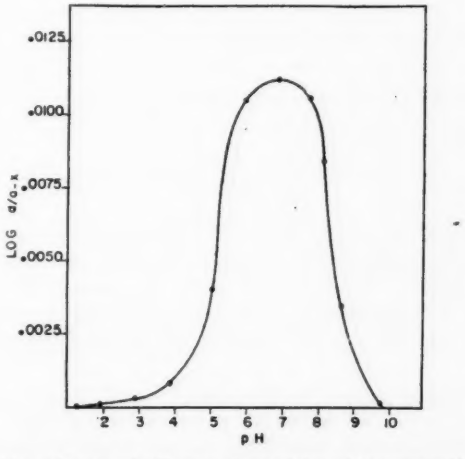

FIG. 5. The influence of pH upon the activity of lactoperoxidase.

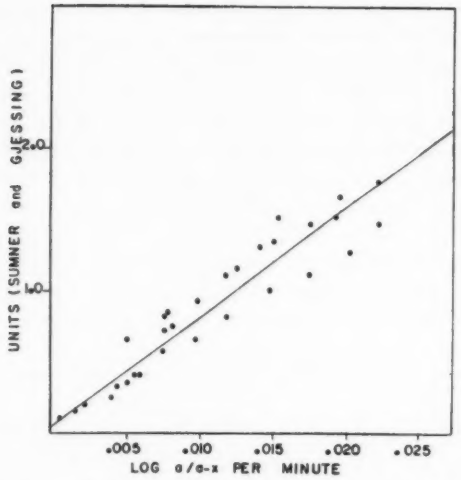

FIG. 6. Comparison of proposed method to the Sumner and Gjessing method.

The results presented in Figure 5 show that the optimum pH for the determination of peroxidase activity, as determined by the *p*-phenylenediamine assay method, is 6.8.

The unit of peroxidase activity most frequently used is P.Z. number.[2] Therefore, it is desirable that the units obtained by the proposed method be translated to P.Z. number for purposes of comparison. Varying concentrations of lactoperoxidase were assayed by both methods. It will be seen that there is close correspondence between the two methods (Figure 6). The coefficient of correlation was + 0.90. The coefficient of variation for the Sumner and Gjessing method was 5.7%, and for the proposed method the coefficient of variation was 6.5%. The values of duplicates run on five different days were used for determining the coefficient of variation. These data were used to derive an equation which would convert lactoperoxidase activity as measured by the proposed method in terms of P.Z. number. The equation is as follows:

$$\text{P.Z.} = \left(\frac{X}{0.0131} + 0.035 \right) \times 10^{-3}$$

In the foregoing equation, X is equal to the change in optical density of *p*-phenylenediamine per minute, under the proposed assay conditions, as determined by least squares. Thus, to assay milk for lactoperoxidase activity, the rate of optical density change of a suitable sample of milk is determined as outlined under *Procedure* and the P.Z. units calculated by the above equation.

[2] The activity of peroxidase, Purpurogallin Number of P.Z., is expressed in terms of the mg. of purpurogallin formed. P.Z. is the number of milligrams of purpurogallin formed per milligram of enzyme preparation when it acts upon 5 g. of pyrogallol in 2 l. of water plus 10 ml. of 0.5% hydrogen peroxide for 5 minutes at 20° C.

Milks of varying lactoperoxidase activity were assayed by both the Sumner and Gjessing method and the proposed method. The results presented in Table 1 show there is good agreement between the two methods.

TABLE 1

Lactoperoxidase activity in milk as determined by both methods

Milk sample	Sumner and Gjessing method	Proposed method
	Units[a]	*Units*[a]
1	0.21	0.21
2	0.24	0.26
3	0.40	0.46
4	0.41	0.48
5	0.58	0.58
6	0.84	0.90
7	1.00	1.03
8	1.18	1.20
9	1.35	1.38
10	1.45	1.51

[a] Represents arithmetic average of duplicate trials.

SUMMARY

The method which has been described possesses the advantages of speed, convenience, and sensitivity. In this method *p*-phenylenediamine is oxidized by lactoperoxidase in the presence of hydrogen peroxide. Under the conditions of the assay, the amount of color developed is proportional to time. The influence of hydrogen peroxide concentration, *p*-phenylenediamine concentration, and enzyme concentration was studied and the optimum conditions were selected.

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COMPARATIVE VALUE OF KENTUCKY BLUEGRASS, KENTUCKY 31 FESCUE, ORCARD GRASS, AND BROMEGRASS AS PASTURES FOR MILK COWS.[1] I. HOW KIND OF GRASS AFFECTED PERSISTENCE OF MILK PRODUCTION, TDN YIELD, AND BODY WEIGHT

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Little is known about the nutritive value of the various grasses used as pasture for milking cows. Efforts in Louisiana and Kentucky to use supplemental feed such as hay, silage, or an extra amount of grain *(9, 10, 12)* in order to keep a high level of milk production while cows are grazing pastures fair to good in quality have met with either little benefit or only moderate improvement in milk production. In short-time trials it was observed, however, that the supplementation apparently was more beneficial when fed to cows grazing Kentucky 31 (tall) fescue or Kentucky 31 fescue and ladino pasture than when fed to cows grazing orchard grass pastures.

In a 3-year trial at the Kentucky Agricultural Experiment Station *(11)* it was found that orchard grass–ladino pasture ranked first in monthly (4-week) persistency of milk production (98.3%), followed in order by Kentucky 31 fescue-ladino (89.2%), Kentucky bluegrass–ladino (87.2%), and a straight stand of Kentucky 31 fescue (81.4%). The straight fescue was fertilized twice yearly with 100 lb. of ammonium nitrate. Under these conditions the fescue alone produced yearly the most TDN per acre (2,327 lb.), whereas the three other pastures yielded about the same, varying from 1,726 to 1,789 lb. per acre. The loss in stands of ladino clover with the fescue and bluegrass, plus the volunteer introduction of a light stand of white clover in the "straight" fescue, probably altered the results secured from the experiment.

Pratt and Haynes *(8)* of the Ohio Station were the first to report experiments with Kentucky 31 fescue as pasture for milk cows and dairy heifers. In 1948 they seeded fescue with alfalfa and ladino clover and secured a stand of mostly fescue and ladino with a scattering of alfalfa. Jersey heifers grazing this pasture in 1949 made gains of 0.2 to 0.4 lb. per head daily as compared with gains of 1.7 lb. daily when grazing bluegrass-ladino pasture or meadow crop mixtures. In a reversal experiment they found that milk cows ate more hay, produced less milk, and lost more weight while grazing Kentucky 31 fescue–ladino than when on the bluegrass-ladino pasture. In another experiment their cows produced more milk while fed in dry lot than while on fescue-ladino pasture.

In a 1953 trial conducted at Hamilton, Ohio, Pratt and Davis *(7)* reported that cows secured 49% more TDN from the bluegrass-ladino than from fescue-

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ladino pasture. They also noted that rapid declines took place in milk production and in body weight.

Experiments conducted by McCullough *et al. (4, 5)* at Experiment, Georgia, showed that temporary forage, ryegrass-oats–crimson clover, as compared with Kentucky 31 fescue for winter pasture resulted in about 30% more milk with an increased TDN intake of 15 to 20%.

Trials were conducted at the Purdue Station *(18)* in 1948, 1949, and 1950, comparing alfalfa–ladino clover–bromegrass pasture with alta fescue-birdsfoot trefoil pasture (5% or less consisted of trefoil). Results showed less yield of milk per acre and a more rapid decline in milk from the pasture containing alta fescue. Work at the Virginia *(2)*, North Carolina *(17)*, Tennessee *(16)*, and South Carolina *(3)* stations all give evidence of high yields of TDN from Kentucky 31 fescue but raise questions about its palatability and its ability to maintain high levels of milk production.

EXPERIMENTAL PROCEDURE

During the fall of 1951, eight 2-acre pasture plots were prepared, fertilized according to indicated soil needs, and seeded to straight stands of grasses. Two plots, each allocated at random, were seeded to Kentucky bluegrass, Kentucky 31 fescue, orchard grass, and Lincoln bromegrass. Satisfactory stands of the grasses with comparable densities resulted. Heaving of the ground as a result of hard freezing during the first winter, however, reduced all stands somewhat, with Kentucky 31 fescue and Kentucky bluegrass apparently having the thinner stands of grass during the 1952 grazing season. Natural thickening of the stands of these two grasses during the next 2 years took place. In contrast, the stands of orchard grass and bromegrass became somewhat thinner, so that at the termination of the experiment the fescue and bluegrass had the thicker stands of grass, as indicated by cover of the ground. Because of drought the stand of orchard grass was greatly reduced during the summer of 1953. During 1954, crab grass contributed much toward the grazing on these orchard grass plots.

Three applications of 100 lb. each of ammonium nitrate (33.5% N) were made during each grazing season, usually in April, July, and September. This was done to secure rapid growth and to compensate for the nitrogen supplied by legumes when present in permanent pastures. In the fall applications of nitrogen fertilizer in 1952 a part of each bromegrass plot (about one-tenth of one and one-half of the other) through an error was not treated. Even though these areas were given special treatment the next spring, there was a decided reduction in the stands of grass as a result of winter killing on these portions of the two plots.

By the use of the "put and take" procedure, test pastures were managed so as to keep all areas grazed to the same degree of closeness and to prevent overgrazing. During times of drought, cows were removed from all areas for short periods, during which a bluegrass–white clover permanent pasture was grazed. During test periods two or more cows were on each plot and the cows were assigned at random to each replicate of the four grasses after first sorting them

into groups of four cows as nearly alike as possible with regard to breed, age, milk production, and stage of lactation.

One cow on each of the eight plots received no grain as a supplement, and these were used as "testers" to determine digestibility of the forage. The results of this phase of the experiment are reported in a subsequent paper. All other cows received grain (14 to 16% protein) fed according to the plan of Morrison *(6)*, which considers milk yield, fat test, weight of cows, and quality of pasture. Adjustments were made every 4 weeks in amounts of grain fed.

Persistency of milk production (FCM) was determined for each 4-week test period by calculating the ratio (as a per cent) between the amount the cows produced during the fourth week and the amount they produced during the week prior to the test period. This persistency was based on all normal lactating cows that remained continuously on the plots for the entire 4-week period. In most cases (all of 1954) the number was two per plot, but in a few cases the data on three or four cows were used in order to get a more accurate appraisal.

The yield of calculated total digestible nutrients (TDN) was kept on each of the eight plots. This was based on uniform procedures *(1)* which consider yield of FCM, body maintenance, and gains or losses in body weight of cows. Body weights were based on 3-day average weights of cows for 3 days prior to and at the end of each 4-week test period. Actual daily milk weights were kept and butterfat tests were made from 2-day composite samples made weekly.

Data secured in this experiment were summarized by use of the analysis of variance as described by Snedecor and Cox *(14)* and Snedecor *(13)*.

EXPERIMENTAL RESULTS

Persistency of milk production. Data were gathered relative to persistency of milk production for three 4-week periods during 1952 and four such periods in both 1953 and 1954.

The 4-week persistency averages for all cows for each of the 3 years are presented in Table 1. The data, presented as averages for cows on each plot, show remarkable consistency among replicates for the various grasses. The analysis of variance indicated very little variation among replicates or among years but highly significant variation among kinds of pasture, indicating that bromegrass, orchard grass, and bluegrass were all significantly superior to fescue in maintaining a high persistency in milk production but that the three grasses did not differ significantly among themselves.

The persistency in milk production of the digestion (nongrain) cows averaged for the 3 years 89.5% on bromegrass, 87.3% on bluegrass, 83.3% on orchard grass, and 75.6% on fescue. In general, the ranking was about the same as for all cows, but there was much more variation from year to year and among replicates, which caused differences to be slightly too small to be statistically significant. The variation found would be in line with expectations with just one cow per plot and with some cows being better able than others to produce at a high level without grain. For example, the performance of No. 609, a large Holstein cow, on the fescue replicate No. 2 for 1953 is of special interest. Her

TABLE 1

Effect of kind of grass pasture on persistency of milk production

Year	Replication	Bromegrass			Orchard grass			Fescue			Bluegrass			Average
		1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	
		(%)												
1952		92.8	93.4		89.7	91.6		72.0	69.7		98.9	90.0		87.3
1953		89.8	90.3		88.4	85.9		70.3	78.8		92.7	92.3		86.1
1954		87.2	91.2		85.3	84.7		75.8	66.9		85.5	90.5		83.4
Average				90.8			87.6			72.2			91.6	85.6

TABLE 2

Influence of kind of grass pasture on body weight changes
3-year summary—weight gain per day per cow

Year	Replication	Bromegrass			Orchard grass			Fescue			Bluegrass			Average
		1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	
		(lb.)												
1952		−0.91	−0.20		−0.14	−0.12		−2.64	−1.35		−0.59	0.48		−0.68
1953		1.48	−0.38		−0.15	−0.56		−0.63	−0.86		0.41	0.45		−0.03
1954		0.48	0.44		−0.11	0.44		−1.70	−2.03		0.60	0.27		−0.20
Average				0.15			−0.11			−1.54			0.27	−0.30

TABLE 3

Yield of TDN per acre per year for grass pastures

Year	Replication	Bromegrass			Orchard grass			Fescue			Bluegrass			Average
		1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	
		(lb.)												
1952		1,416	1,916		1,417	1,161		640	785		1,408	1,136		
1953		2,498	2,050		1,837	2,037		2,261	2,271		2,329	2,282		
1954		2,863	2,961		2,489	2,754		2,504	1,939		2,952	2,899		
Average				2,284			1,949			1,733			2,168	2,034

persistency continued high, averaging 90.2%, yet she suffered an unusually large loss in body weight, totaling 254 lb., during the experiment.

How well the four grasses prevented rapid declines in milk production during the various months of the year was investigated. The data studied were the average percentages for weekly decline for each kind of grass over the 3-year period. It was necessary to use the weekly measurement because most of the 4-week test periods on which the data in Table 1 are based included portions of 2 months.

In comparing the four grasses, it was found that beginning in April and continuing through September the cows grazing fescue showed the heaviest average weekly decline for each month. Only during March, April, and July did the decline stay around the approximately 2% weekly decline *(15)* expected in a normal lactation. Bromegrass, orchard grass, and bluegrass had similar seasonal trends.

Body weight changes. Changes in body weight for all cows, when reduced to average daily gain in weight per cow for each plot and for each year, are as shown in Table 2. In general, body weights of cows were not materially changed while the animals were grazing bromegrass or orchard grass. They tended to show some gain (0.27 lb. per cow per day) while on bluegrass, and there were highly significant losses, averaging 1.54 lb. daily per cow, while the cows were grazing fescue. As with milk persistency the bromegrass, orchard grass, and bluegrass pastures did not show significant differences, but all of them indicated that they were significantly superior to the fescue pasture.

Yield of TDN per acre. As shown in Table 3, the yields of calculated TDN based on milk yield, body maintenance, and body weight changes *(1)* show remarkable uniformity as between replications but much variation among years and kinds of pasture. Significant differences ($P < 0.05$) were found among the average yields of the four kinds of pasture, with 2,284 lb. yearly per acre for bromegrass being highest and 2,168 lb. for bluegrass and 1,949 for orchard grass being only slightly less. Bromegrass and orchard grass both out-yielded fescue significantly, as the 3-year average of fescue was only 1,733 lb. per year. A portion of the low yield for fescue was due to its poor showing the first year when the stand was thin, as was also true with bluegrass. Of greater importance, however, was the reduction in TDN yield for fescue caused by the loss in body weight (see Table 2) that took place for all years but which was unusually heavy in 1952 and 1954. Most of the time the fescue pastures carried more cows than did the other pastures, yet body weight losses and rapid decline in milk production combined to reduce their calculated yields of TDN per acre.

The bromegrass yields probably would have been higher had not the error occurred (as described under experimental procedure) relative to fertilizing portions of each replicate of this grass in the fall of 1952.

SUMMARY

Eight 2-acre plots were seeded in the fall of 1951 to two pastures each of Kentucky bluegrass, Kentucky 31 fescue, orchard grass, and Lincoln bromegrass.

At seeding time the eight areas were all fertilized alike and in line with needs as indicated by soil tests. Also, three times each year the pastures were treated with 100 lb. of ammonium nitrate (33.5% N) per acre. These pastures were then grazed for 3 years by dairy cows, and comparisons were made relative to their yield of TDN yer acre and their ability to maintain high persistency of milk production and body weights of the cows.

The 4-week persistency in milk production averaged for 3 years 91.6% for bluegrass, 90.8% for bromegrass, 87.6% for orchard grass, and 72.2% for fescue. The cows used in digestion studies (nongrain) had similar averages but more variability among replicates.

Body weight gains based on the 3-year daily average per cow were 0.27 lb. for bluegrass, 0.15 lb. for bromegrass, –0.11 lb. for orchard grass and –1.54 lb. for fescue. Yields of TDN yearly per acre averaged 2,284 lb. for bromegrass, 2,168 lb. for bluegrass, 1,949 lb. for orchard grass and 1,733 lb. for fescue. The fescue actually carried more cows per acre, and its low rating on TDN yield was caused largely by the rapid decline in milk production and the heavy losses in body weight by cows grazing it.

The 3-year trial indicated only minor and insignificant differences among Kentucky bluegrass, Lincoln bromegrass and orchard grass, when highly fertilized and used as pasture crops for milk cows. Kentucky 31 fescue, however, failed to equal these grasses, particularly when compared with them on ability to maintain high milk production and body weight of milk cows.

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COMPARATIVE VALUE OF KENTUCKY BLUEGRASS, KENTUCKY 31 FESCUE, ORCHARD GRASS, AND BROMEGRASS AS PASTURE FOR MILK COWS. II. EFFECT OF KIND OF GRASS ON THE DRY MATTER AND CRUDE PROTEIN CONTENT AND DIGESTIBILITY AND INTAKE OF DRY MATTER[1]

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Very little data are available concerning the relative nutritive value of pasture grasses for milking cows. A number of workers *(2, 3, 19)* have shown that the crude protein content and the digestibility of pasture grasses are highest during the early part of the pasture season and decline as the season advances, with a further decline or increase in late summer or early fall, depending on moisture and temperature conditions. Swift *et al.* in 1950 *(17)* and 1952 *(16)* in a study of the nutritive value of forages reported that Kentucky bluegrass was the highest of six forages studied, including bluegrass, orchard grass, and bromegrass, in metabolizable energy, digestible energy, and total digestible nutrients. Fuelleman and Burlison *(5)* found that under grazing conditions, on the basis of growth and consumption, bromegrass ranked first, bluegrass and orchard grass about equal, and reed canary grass a poor fourth. Forbes and Garrigus *(4)* observed that wether lambs consumed considerably more orchard grass than Kentucky 31 fescue. Miller *et al. (9)*, using dry forage, in 1955 reported that the ether extract and crude protein fractions of tall fescue were more digestible than those of orchard grass.

Thompson and Holdaway *(18)* found little difference between an alfalfa–orchard grass mixture and a ladino–orchard grass mixture, whereas cows grazing a ladino–Kentucky 31 fescue mixture produced significantly less milk than those having access to the other mixtures. Pratt and Haynes *(11)* found that heifers grazing Kentucky 31 fescue made gains of considerably less magnitude than heifers grazing other grasses. Milk production yields and losses in body weight indicated that a Kentucky 31 fescue pasture was inferior to a Kentucky bluegrass pasture. Seath *et al. (13)* found that a straight stand of fescue was inferior to a bluegrass-ladino, fescue-ladino, or orchard grass–ladino pasture for milking cows even though the fescue was fertilized with 66 lb. of nitrogen yearly. In a subsequent study *(14)*, it was found that a Kentucky 31 fescue pasture was inferior to an orchard grass, bromegrass, or bluegrass pasture in respect to persistency of milk production, body weight gains or TDN yield. The data reported herein were collected from the same experiment.

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In the present study an attempt was made to determine the nutritive value of four grasses common to Kentucky in an effort to explain why these grasses differ in their value as forages for milk cows.

EXPERIMENTAL PROCEDURE

Eight pasture plots of 2.0 acres each were seeded in August, 1951, to Kentucky bluegrass, Lincoln bromegrass, orchard grass, and Kentucky 31 fescue. The method of seeding, fertilization rates, and management practices were described earlier *(14)*.

Data were collected on the digestibility and dry matter intake of the various grasses, one lactating dairy cow being used on each plot. One Holstein was assigned to one replicate and one Jersey cow to the other replicate for each species of grass and received no supplemental feed. Digestion trials were conducted every 2 weeks during the 1952, 1953, and 1954 pasture seasons except when the cows were not on the test areas owing to the lack of forage. The digestibility of forage dry matter and protein was determined by the Reid fecal chromogen technique *(12)*. The Evelyn Photoelectric Colorimeter used in this study was standardized with the instrument by Reid *et al. (12)*. Forage samples were taken every 2 weeks to correspond to the digestion trials and analyzed for dry matter and protein *(1)*. During the second and third years the digestion studies included the estimation of the intake of forage dry matter by combining the fecal chromogen *(12)* and chromic oxide *(6)* techniques. Fifteen grams of chromic oxide was administered to each cow daily by capsule. The chromic oxide was given 10 days before samples of feces were taken. The method of fecal collection varied from year to year. During 1952, a single "grab" sample of feces was taken every 2 weeks. During 1953, fecal samples were taken twice daily for three consecutive days every 2 weeks, and during 1954 samples were taken twice daily for five consecutive days every 2 weeks. Fecal samples were taken at 6:00 A.M. and 4:00 P.M. The sampling procedure was varied as the new information regarding the use of the fecal chromogen and the chromic oxide techniques became available.

RESULTS AND DISCUSSION

The content of dry matter and crude protein of the various grasses is presented in Table 1. Statistical analyses *(15)* of these data show that the dry matter content of bluegrass was significantly higher than that of any of the

TABLE 1

Effect of the kind of grass on the forage content of dry matter and crude protein (per cent—dry matter basis—yearly average)

Year	Dry matter				Crude protein			
	Brome	Orchard	Fescue	Bluegrass	Brome	Orchard	Fescue	Bluegrass
1952	31.1	30.9	31.0	40.2	23.7	21.0	18.5	17.9
1953	22.6	25.2	24.0	32.4	27.2	24.1	22.4	19.7
1954	26.3	26.9	26.2	35.8	26.0	22.8	23.1	20.1
Av.	27.0	27.7	27.1	36.1	25.6	22.6	21.3	19.2

other grasses. The reason for this was apparently the inherent characteristics of the grass, since all grasses were fertilized and managed alike. Even though the grasses varied in dry matter content from year to year, that of bluegrass was the highest each year of the study.

Analysis of the crude protein content data indicated that the protein content of bromegrass was significantly higher than that of any of the other grasses and that the content of orchard grass was significantly higher than that of bluegrass. The superiority of bromegrass in this respect was evident throughout the study, for it ranked the highest each of the 3 years. Even though the protein content of bluegrass was considerably below that of the other grasses, seemingly this should not be a limiting factor as to its nutritional value, since the same consumption of this grass as of the other grasses would represent an intake of crude protein in excess of the accepted minimum protein requirements for lactating cows *(10)*. As was pointed out previously *(14)*, these grasses were treated with 100 lb. of nitrogen per year, which probably accounts for the relatively high protein content of all grasses *(8)*.

TABLE 2

Effect of kind of grass on apparent digestibility of forage dry matter and crude protein (per cent—yearly average)

Year	Dry matter				Crude protein			
	Brome	Orchard	Fescue	Bluegrass	Brome	Orchard	Fescue	Bluegrass
1952	62.8	65.1	63.3	61.3	75.4	72.2	72.1	69.4
1953	68.6	69.3	68.2	67.8	81.6	78.6	77.1	73.7
1954	66.6	67.0	67.5	65.8	74.2	72.4	75.2	70.8
Av.	66.0	67.1	65.7	65.8	77.1	74.4	73.9	71.7

In Table 2 the apparent digestibility of dry matter and crude protein of the various grasses is shown. It was found that the digestibility of orchard grass was significantly higher than that of bromegrass, fescue, or bluegrass, and the digestibility of bromegrass and fescue was significantly higher than that of bluegrass. Even though the differences between grasses were significant, it is doubtful if any of the differences were of a magnitude to be of any great importance from a nutritional viewpoint since all of the values were rather high.

McCullough *et al. (8)* observed that the dry matter digestibility of fescue was approximately 13 percentage points below that of a temporary pasture composed of oats, rye grass, and crimson clover. Probably some difference in nutrient digestibility would exist between a temporary pasture as used by these workers and a permanent pasture, but probably another factor was largely responsible for the decreased digestibility of the fescue pasture. In their study, hay and grain were fed in addition to the forages, and the proportion of hay and grain fed was not the same with each type of forage. The cows grazing fescue received 18% of their total dry matter intake from hay, 22% from grain, and 60% from pasture, whereas the cows grazing the temporary pasture received 9% from hay, 16% from grain, and 75% from pasture. One would expect the cows fed the fescue forage ration to have a lower dry matter digestibility than the temporary

pasture ration, since the temporary pasture ration contained a higher percentage of nutrients from pasture and a lower percentage from hay than did the fescue pasture, even though the cows grazing fescue obtained a higher percentage of nutrients from grain.

In a later report McCullough *(7)* concluded that the low digestibility of fescue forage, 64% as compared with 75% for a winter temporary pasture, was its most significant limitation as a forage for milking cows. It was further suggested that fescue may contain an inhibitory factor as far as milk production is concerned. Data obtained in this study certainly do not indicate that the dry matter digestibility of fescue is the limiting factor as to its milk-producing qualities. For example, the dry matter digestibility of bluegrass was found to be lower than that of fescue but, as reported previously *(14)*, the cows on bluegrass had a much higher persistency of milk production than those on fescue. It is doubtful if one can expect the nutritional qualities of any permanent pasture grass to be as high as those of a temporary pasture except at specific times during the grazing season.

As shown in Table 2, the crude protein digestibility of bromegrass was considerably higher than that of the other grasses. The digestibility of orchard grass and fescue was significantly higher than that of bluegrass, but the difference between the digestibility of orchard grass and fescue was not significant. Apparently the high protein content and high digestibility of this protein in bromegrass contribute greatly to its nutritional value. These data indicate that both the protein content and the digestibility of the protein in bluegrass are lower than those of the other grasses studied. This fact is not readily explained, but this does not appear to affect the milk-producing qualities of bluegrass *(14)*.

TABLE 3

Effect of kind of grass on daily intake of forage dry matter (lb/1,000 B.W., yearly average)[a]

Year	Grass			
	Brome	Orchard	Fescue	Bluegrass
1953	26.8	26.5	22.7	27.8
1954	18.1	22.1	17.3	18.8
Av.	22.4	24.3	20.0	22.3

[a] Data not obtained in 1952.

As can be observed in Table 3, the kind of grass being grazed had a pronounced effect on the intake of dry matter when stated in terms of pounds of intake per 1,000 lb. of body weight. Statistical analysis of these data indicated that the differences between grasses were approaching significance at the 5% level of probability. The relationship between the intake of dry matter of the various grasses and the persistency of milk production *(14)* of these cows appears to be rather high, since bromegrass, orchard grass, and bluegrass all had similar estimates of dry matter intake and had similar persistency of milk production values. Both the intake of dry matter and the persistency of milk production

for cows grazing fescue were below those for cows grazing any of the other grasses.

Based on the several measurements of nutritional value employed in this study, it would appear that the lack of sufficient dry matter intake of fescue is its most limiting factor as regards its milk-producing properties. However, these data also indicate that another factor(s) may be involved. For example, the cows grazing orchard grass had a lower persistency rating than the groups on bromegrass, orchard grass, or bluegrass but had the highest intake of dry matter. The persistency rating and dry matter intake estimates for both bromegrass and bluegrass are in very good agreement. It should be pointed out that none of the differences between these three grasses in either the measurement of dry matter intake or persistency rating were significantly different; therefore, these small differences could account for the discrepancy with regard to orchard grass.

Figure 1 shows the trend in the nutritive value of the forages throughout the pasture season. The values for all grasses were combined, since in most instances the trends were similar for all grasses. All grasses showed similar trends in regard to dry matter and protein content and dry matter and protein digestibility. The dry matter content of the grasses tended to be rather low during

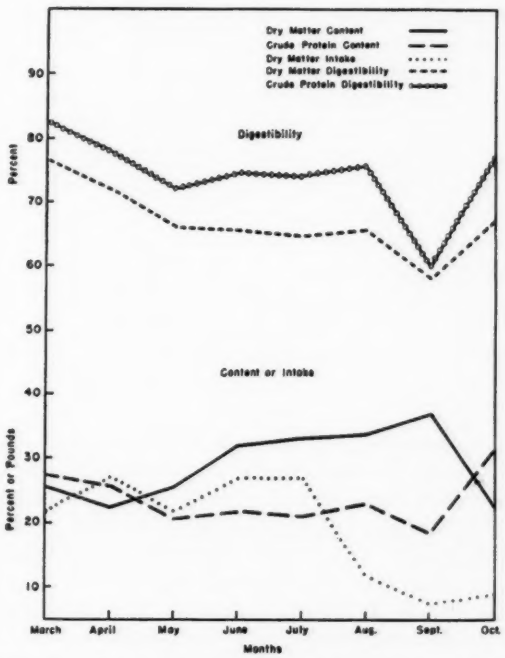

FIG. 1. Seasonal trends of dry matter and crude protein content, dry matter and crude protein digestibility, and intake of dry matter (lb/day/cow/1,000 lb. of B.W.) of all grasses when combined.

March, April, and May, high in the midsummer months, and again rather low in October. It should be pointed out that the number of observations for either early spring or for fall grazing were not so numerous as during midsummer months. For example, the cows grazed the pastures only in March during the 1953 grazing season and during September and October in 1954. This means that in these months the values presented are the results from just one year's observations. The protein content of the grasses followed an inverse relationship, with dry matter content being extremely high in early spring and fall and medium-low during midsummer. The digestibility of both dry matter and crude protein followed very closely the patterns presented for the grasses content of these constituents except in a negative manner. A low dry matter or high protein content resulted in a high digestibility coefficient for each nutrient.

The consumption of dry matter remained at a medium-high level for all grasses, with the consumption of fescue being slightly less than that of the three other grasses, from March through July. Thereafter, the intake of all grasses declined considerably during the months of August, September, and October with the exception of fescue, which remained relatively high through August and then declined. The medium-high dry matter intake of cows grazing fescue in August is unexplainable at the present time and cannot be correlated with the milk production of cows during this month. Data reported previously *(14)* showed that the persistency ratings of cows grazing fescue was the lowest for any grass during the month of August; yet the intake of dry matter of this grass during this period was the highest of any grass. During other times of the pasture season a very close relationship existed between dry matter intake by the cows and their persistency of milk flow. Cows grazing fescue had a high persistency of milk flow during very early spring, which then dropped to a low level the remainder of the grazing season *(14)*. Dry matter intake of this grass was highest of all grasses during March but then declined to a suboptimal level the remainder of the year with the exception of the unexplainable value obtained during August. This inconsistency indicates again that a factor(s) other than the consumption of forage may be involved in the lower persistency of milk production of cows grazing fescue.

SUMMARY

A 3-year study was made to determine the nutritional value of four grasses—Lincoln bromegrass, orchard grass, Kentucky 31 fescue, and Kentucky bluegrass for lactating dairy cows. Digestibilities of dry matter and crude protein were made by the fecal chromogen method, and estimates of dry matter consumption were made by a combination of the fecal chromogen and chromic oxide techniques.

The dry matter content of bluegrass was significantly higher than that of the other grasses. The crude protein content of bromegrass was significantly higher than that of all other grasses, and the content of orchard grass was significantly higher than that of bluegrass. The protein content of the grasses averaged 25.6, 22.7, 21.4, and 19.2% for bromegrass, orchard grass, fescue, and bluegrass, respectively. The digestibility of dry matter in orchard grass was

higher than that of the other grasses, but since all grasses were high in this respect it is doubtful if much significance should be attached to this observation. The crude protein digestibility of bromegrass was considerably higher, and the protein digestibility of bluegrass lower, than that of the other grasses.

The daily intake of dry matter by cows grazing bromegrass, orchard grass, and bluegrass was essentially the same, averaging, respectively, 22.5, 24.3, and 23.3 lb. per 1,000 lb. of body weight. The intake of fescue was somewhat below that of these three grasses, averaging only 20.0 lb. daily. These differences approached significance at the 5% level of probability. Apparently the reduction in dry matter intake of cows grazing fescue is a primary reason why it had been observed that cows grazing this grass did not have a high persistency of milk flow *(14)*. Data were also presented to indicate that another factor(s) may be involved.

The seasonal trend was similar for all grasses, with the dry matter content being low in the early spring and fall and crude protein content high during these periods. Dry matter digestibility was high when the content of the grasses was low, and protein digestibility high when the protein content of the grass was high. The intake of dry matter remained at a high level from March through July but decreased to a suboptimal level in August and September for all grasses except fescue, which held a medium-high intake during August.

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CHEMICAL DETERMINATION OF 17-HYDROXYCORTICOSTEROIDS IN THE BLOOD OF CATTLE AND SOME INDICATIONS OF ITS PHYSIOLOGICAL SIGNIFICANCE[1]

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Querido *et al. (19)* have stated that the level of blood 17-hydroxycorticosteroids is the best criterion of adrenal cortical function. Information in regard to the blood levels of these substances in cattle is lacking, although a review of the literature by Dorfman and Ungar *(6)* lists 27 steroids that have been isolated and identified in bovine adrenal tissue. Hechter *et al. (10)* obtained both corticosterone and 17-hydroxycorticosterone from cattle blood, but the method involved extensive column and paper chromatographic fractionations and as such was not well suited to routine quantitative analysis.

A number of chemical assays have been proposed for determining 17-hydroxycorticosteroids in biological fluids. One group of assays *(2, 3, 4, 5, 9, 13, 22, 24)* is dependent on the reducing property of the side chain at carbon 17 or in the same manner on the reducing property of any nonsteroidal contaminant present in the final extract. The results obtained with the reducing assays are reliable only to the extent that the extraction and fractionation procedures employed are specific for the 17-hydroxycorticosteroids.

Another type of chemical assay that has been used rather widely is the periodic acid oxidation of the side chain at carbon 17 to produce formaldehyde. The formaldehyde is then measured by various colorimetric procedures *(7, 11, 12)*. Here again, as in the reducing assays, there is a lack of specificity since many nonsteroidal substances, which could very possibly contaminate a final extract, will produce formaldehyde under these conditions.

This paper presents a quantitative method for the determination of total free 17-hydroxycorticosteroids in the blood plasma of dairy cattle, suitable for routine analysis. A preliminary report on the method *(20)* has already been presented. This method utilizes a micro-modification of the Porter-Silber color reaction, dependent on the formation of phenylhydrazones in an acid solution, and is specific for 17-21-dihydroxy-20-ketones *(18)*. Nelson and Samuels *(16)* have determined 17-hydroxycorticosteroid levels in human blood and plasma by using a modification of this same reaction. Other workers also have utilized this reaction to study relationships between plasma 17-hydroxycorticosteroid levels and various clinical and experimental conditions in the human *(1, 8, 15, 21)*.

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METHOD

Reagents:

1. Ethyl acetate, C. P., redistilled.
2. Hexane, C. P., redistilled.
3. Sodium chloride, C. P.
4. Methanol, C. P., redistilled.
5. Dilute sulfuric acid, 310 ml. sulfuric acid C. P., and 190 ml. distilled water.
6. Phenylhydrazine HCl, C. P., recrystallized from ethanol four times.
7. Phenylhydrazine—dilute sulfuric acid reagent. 57 mg. of reagent 6 plus 50 ml. reagent 5. Make up fresh each day.

Extraction. Collect jugular vein blood[3] under aseptic conditions and within 2 hours after collection, centrifuge and separate the plasma. Place the plasma in glass-stoppered flasks and chill to 4° C. Add 6 g. of sodium chloride to 80 ml. of chilled plasma and transfer to a prechilled, 250-ml. separatory funnel. Extract for precisely 30 seconds with 80 ml. of cold ethyl acetate (4° C.). Draw off the plasma, rechill, and extract with another 80 ml. of cold ethyl acetate in a prechilled separatory funnel. Combine the ethyl acetate extracts and wash with 3 × 10 ml. portions of saturated sodium chloride solution, 5 × 10 ml. portions of cold 0.1 *N* sodium hydroxide solution, and finally with 10-ml. portions of distilled water until the wash is neutral. Allow the ethyl acetate extract to stand over an excess of anhydrous sodium sulfate for 10 minutes and then filter and measure the ethyl acetate recovered. The plasma equivalent of the recovered ethyl acetate is determined by dividing the volume by two, since two volumes of ethyl acetate were used in the original extraction. Reduce the extract to dryness with the aid of a gentle stream of air in a water bath maintained at 50° C. Transfer the methanol soluble portion of the residue to a centrifuge separatory funnel with the aid of 3 × 5 ml. portions of 80% methanol. Partition the methanol three times with 15-ml. portions of hexane, using gentle centrifugation each time to aid in separation of the layers. Discard the hexane and transfer an aliquot of the methanol phase (adjusted to contain 20 to 30 ml. of plasma equivalent) to each of two glass-stoppered test tubes. There is a negligible loss of steroid incurred during the methanol-hexane partition. Reduce the extract to dryness with the aid of a gentle stream of air in a water bath maintained at 50° C.

Phenylhydrazine assay. Dissolve the dried steroid in each of the two tubes with 0.2 ml. of methanol. Use one tube as an acid plasma blank and the other as a reaction tube. To the acid plasma blank add 0.8 ml. of dilute sulfuric acid. To the reaction tube add 0.8 ml. of the phenylhydrazine-dilute sulfuric acid reagent. Shake to obtain thorough mixing and place the tubes in a water bath maintained at 55° C. for 20 minutes. Remove, cool in tap water for 2 minutes, and transfer to cuvettes (10 mm. light path) and determine the optical density in a Beckman Model B spectrophotometer at 410 mμ.

[3] Anticoagulant is composed of 24 g. of ammonium oxalate, 16 g. of potassium oxalate in 1 l. of water. Citrate anticoagulant is not satisfactory as it interferes with the assay. Five ml. of anticoagulant dried down in a flask is sufficient for 100 ml. of blood.

To accommodate the volumes used in the reaction, a special plate was designed to attach to the side of the absorption cell holder. The plate cuts the height of the incident light to 5 mm., and the cuvettes containing 1.0 ml. of solution are then centered in this area, retaining the 10 mm. light path, with no limitation on the width of the incident light except those inherent in the instrument.

EXPERIMENTAL PROCEDURE

Ringbom analyses. In order to determine the lowest amount of 17-hydroxycorticosterone (Compound F) which could be properly accommodated by the 1.0 ml. phenylhydrazine reaction, a Ringbom plot was prepared *(25)*. An absolute 1% photometric error is assumed in this presentation and would result from the limits of reproducibility of the particular instrument being used. The reaction was studied over the range of 0.5 γ through 10.0 γ of standard material, as shown in Figure 1. As indicated by the point of tangency, the range over which a relative analysis error of 5% or less may be expected is from 2.0 to 10.0 γ.

Standard curve. To test both the linearity of the dose-response curve and the repeatability inherent in the phenylhydrazine reaction, a standard curve was made that would fall within the range limitations established by the Ringbom plot. Tubes were prepared containing 2.0, 4.0, 6.0, 8.0, and 10.0 γ of 17-hydroxycorticosterone. Six series were run with each dosage in duplicate in each series;

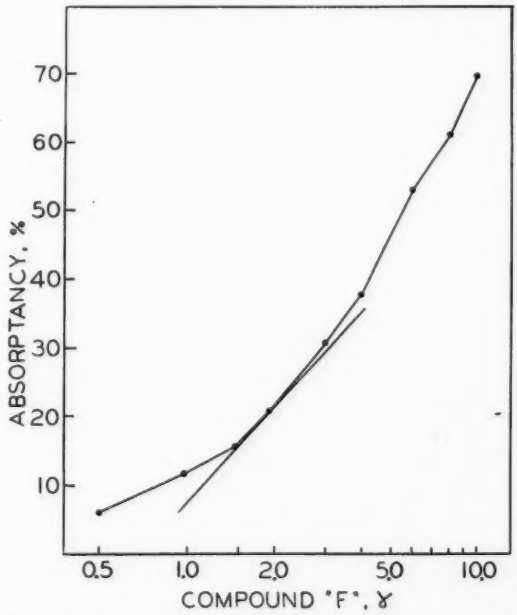

FIG. 1. A Ringbom plot of the phenylhydrazine reaction showing that 2-10 γ of 17-hydroxycorticosterone may be assayed with a relative analysis error of 5% or less.

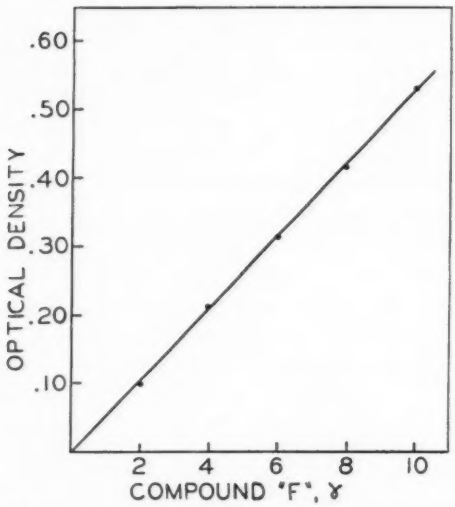

FIG. 2. Phenylhydrazine standard curve using 17-hydroxycorticosterone (free alcohol) as the standard material.

thus each point in Figure 2 is the mean of 12 determinations. It will be noted that the data are strictly linear over the dosage range covered.

An analysis of variance of the data (Table 1) showed that the series effect was not significant, indicating good repeatability of the reaction. When series, series × dosage, and residual effects were all included in the error term, a coefficient of variation of 4.47% and a standard error of a single observation of 0.258 γ in terms of 17-hydroxycorticosterone were obtained. A regression equation was also derived by using the least squares method, and this equation was used in calculating plasma corticoid values.

Absorption spectra. A comparison of the absorption spectra obtained with 17-hydroxycorticosterone and with a plasma extract (28.0 ml. of plasma equivalent) is shown in Figure 3. Peak absorption in both instances occurs at 410 mμ and is specific for the 17,21-dihydroxy-20-ketone grouping.

Efficiency of extraction. The efficiency of the extraction procedure was assessed by adding 17-hydroxycorticosterone (free alcohol) to plasma and calculating recovery. Blood samples were obtained and centrifuged, and the plasma

TABLE 1

Analyses of variance of the phenylhydrazine standard curve data

Variance source	Degrees of freedom	Mean square
Total	59	
Dosage	4	0.338118*
Series	5	0.000429
Dosage × series	20	0.000232
Residual	30	0.000133

* Highly significant effect ($P < 0.01$).

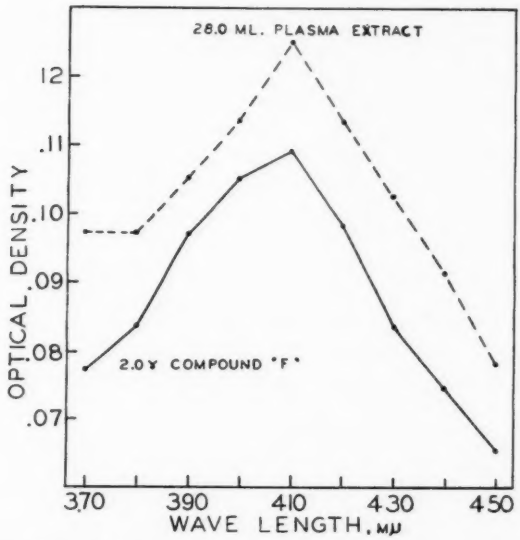

FIG. 3. A comparison of absorption spectra obtained with a plasma extract and 17-hydroxycorticosterone in the phenylhydrazine reaction.

was separated. Samples of plasma (80 ml.) were placed in each of two glass-stoppered 125-ml. flasks. One of the samples served as a control, and 17-hydroxycorticosterone was added to the other. The samples were then placed in a refrigerator and held at 4° C. overnight. Extraction and assay were accomplished the next day. A mean recovery of 81.0% of the added steroid was obtained with no significant variation in recovery over the range of steroid concentrations studied (Table 2). This performance agrees well with the results of Nelson *(15)*, who reported a mean recovery of 80% of added 17-hydroxycorticosterone from human plasma.

Effect of ACTH on plasma 17-hydroxycorticosteroids. To assess the physiological significance of the plasma extract fraction, six dairy cows were injected

TABLE 2

Efficiency of extraction of 17-hydroxycorticosterone from plasma

Plasma sample No.	F added	F recovered	Recovery
	(γ %)	*(γ %)*	*(%)*
1	17.5	13.88	79.3
2	12.5	10.26	82.1
3	10.0	7.70	77.0
4	10.0	8.28	82.8
5	10.0	8.47	84.7
6	7.5	6.37	84.9
7	5.0	3.89	77.8
8	5.0	3.97	79.5
Mean			81.0

TABLE 3

Increase in plasma 17-hydroxycorticosteroids 2 hours after the intramuscular injection of 600 A.V.U. of ACTH

Cow No.	Initial F	2-hour F	Increase
	(γ %)	(γ %)	(%)
1	17.62	29.79	67
2	12.85	29.74	116
3	9.64	19.73	105
4	7.00	14.38	105
5	5.10	13.80	170
6	2.98	7.74	159
Mean	9.20	18.86	120

intramuscularly with 600 Armour Veterinary Units of ACTH. Blood samples were collected from the animals immediately before and 2 hours after the ACTH administration. A mean increase of 120% in the level of circulating 17-hydroxycorticosteroids was obtained in these trials (Table 3) as a consequence of ACTH administration. These results are very similar to those in the human reported by Nelson *et al. (17)*, who found a 125% increase in the level of circulating 17-hydroxycorticosteroids 2 hours after the intramuscular injection of 25 mg. of ACTH.

Plasma 17-hydroxycorticosteroid levels in normal dairy cows. To further assess the physiological significance of the plasma extract fraction, blood samples were obtained from 23 dry, pregnant cows within the 6-week period prior to parturition and from 20 milking, nonpregnant cows within the 6-week period after parturition. The plasma 17-hydroxycorticosteroid values for the dry, pregnant group ranged from 6.90 to 17.62 with a mean value of 9.77 γ %, whereas the values for the milking, nonpregnant group ranged from 2.14 to 8.40 with a mean of 4.58 γ % (Table 4). The increase in plasma 17-hydroxycorticosteroid levels accompanying pregnancy is in agreement with that in the human reported by Gemzell *(8)*, who found 100% increase in the plasma steroid levels during pregnancy.

DISCUSSION

The micro-modification of the phenylhydrazine reaction used in this investigation has several very favorable aspects. The specificity of the reaction is par-

TABLE 4

Comparison of plasma 17-hydroxycorticosteroid values between pregnant, dry cows and milking, nonpregnant cows

Animal group	No. of animals	Plasma 17-hydroxycorticosteroids (γ %)			
		Mean	Standard error of mean	Standard deviation	Range
Pregnant, dry, within 6 weeks of parturition	23	9.77[a]	0.60	2.89	6.90-17.62
Lactating, nonpregnant, within 6 weeks after parturition	20	4.58[a]	0.36	1.61	2.14- 8.40

[a] Highly significant difference between the means ($P < 0.01$).

ticularly desirable when applied to extracts of biological fluids. Of 14 steroids tested by Porter and Silber *(18)*, only those containing the 17,21-dihydroxy-20-ketone grouping reacted to produce solutions exhibiting an absorption maximum at 410 mμ. These steroids did not produce appreciable color in sulfuric acid alone. Silber and Porter *(23)*, in a further examination of this reaction, determined that steroids which bear the 21-hydroxy-20-ketone grouping but not the 17-hydroxyl function, such as corticosterone, reacted with the reagents but the absorption peaks for these substances occurred at 340 to 360 mμ. Very little absorption was evident at 410 mμ. Nitrogen analysis of purified products from the reaction of phenylhydrazine with cortisone and 17-hydroxycorticosterone indicates that the color is due to the formation of bisphenylhydrazones.

It would appear that certain specie differences are involved in regard to the amount and types of chromogenic material found in blood or plasma. The method of Nelson and Samuels *(16)* proved adequate for the determination of 17-hydroxycorticosteroids in human plasma. This method was not found satisfactory for the determination of these substances in dairy cattle plasma in this investigation. There appears to be considerably more nonspecific chromogenic material capable of reacting with dilute sulfuric acid in the plasma of cattle. This material was not removed by the method of Nelson and Samuels *(16)* and caused extremely high background absorption in the phenylhydrazine assay such that the absorption maximum at 410 mμ due to the steroid was entirely obliterated.

The use of a cold ethyl acetate extraction for short periods of time is a departure from the usual means of removing 17-hydroxycorticosteroids from biological fluids. Meyer *(14)* pointed out the advantages of using ethyl acetate under these conditions. A number of organic solvents and solvent mixtures were tested by extracting 40 ml. of whole citrated blood. The solvents were rated by examining the ratio of the weight of the cortisone recovered and the weight of the total residue. Ethyl acetate was found to be the most satisfactory of those investigated in that it removed cortisone efficiently and at the same time removed less extraneous material.

The method proposed in this investigation should prove useful in assessing adrenal cortical physiology in dairy cattle as shown by plasma steroid response to ACTH and also by differences exhibited in plasma steroid levels of pregnant nonlactating and lactating nonpregnant animals.

The recovery of standard material compares very favorably with that obtained by Nelson *(15)*. The specificity and sensitivity of the 1.0 ml. phenylhydrazine reaction are particularly advantageous when applied to relatively crude plasma extracts. The extracts resulting from this method contain no more nonspecific chromogenic material capable of interfering with the phenylhydrazine assay than those resulting from a more tedious silica gel chromatographic fractionation procedure.

SUMMARY

A method for the chemical determination of 17-hydroxycorticosteroids in the plasma of dairy cattle was developed and evaluated. The method employs the

extraction of cold plasma by cold ethyl acetate and assay of the steroids by using the Porter-Silber color reaction. The efficiency of the extraction procedure was judged to be 81% as determined from experiments involving the recovery of steroid added to plasma. A mean increase of 120% in plasma 17-hydroxycorticosteroids was obtained 2 hours after the intramuscular administration of 600 Armour Veterinary Units of ACTH to six cows. Plasma 17-hydroxycorticosteroid values for 23 dry, pregnant cows ranged from 6.90 to 17.62 with a mean of 9.77 γ %. Similar values for 20 milking, nonpregnant cows ranged from 2.14 to 8.40 with a mean of 4.58 γ %. These experiments indicate that the method has considerable physiological significance in assessing adrenal cortical function in dairy cows.

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STRATIFICATIONS AND KINETIC CHANGES IN THE INGESTA OF THE BOVINE RUMEN

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In recent years several groups studying rumen function from different approaches have observed an apparent heterogeneity in rumen ingesta. MacDonald *(8)* observed that the ammonia concentration of samples taken at the same time from different locations in the organ varied considerably but found no consistency in the localization of concentration. Pearson and Smith *(13)*, on the other hand, observed a higher concentration of nitrogen in samples taken from the top ingesta than in samples from the bottom. Even cursory examination of rumen ingesta from fistulated animals reveals that, in general, coarse particles of hay tend to accumulate in the top of the organ while the ingesta in the bottom are relatively liquid. A similar layering of rumen ingesta is frequently seen in the laboratory, where feed particles often rise to the top of the vessel and protozoa collect in the bottom. This spontaneous separation has been utilized by McNaught *et al. (9)* in preparing relatively pure samples of rumen protozoa and bacteria for use in determining the biological value of their proteins.

A marked stratification was observed in the ammonia concentration of rumen ingesta from two fistulated steers on two different rations over several 12-hour test periods in this laboratory, and experiments were conducted in an attempt to appraise the extent to which other fermentation products, feed components, and fermentation processes might show similar localization. A clearer understanding of the nature and magnitude of such stratifications was believed necessary to the intelligent selection of sampling locations in studies of rumen function and to the eventual development of a reasonable description of the function of the organ.

The work to be reported here does not constitute a new theory of rumen physiology but does provide some of the data needed to define rumen conditions. The data demonstrate rumen processes that have already been suggested by authors who are concerned with rumen fermentation in relation to animal physiology *(12)*.

EXPERIMENTAL PROCEDURE

Kinetic studies of stratification in fistulated animals. Grade 2-year-old beef-type steers with some dairy blood (animals A, B, and C) were fistulated and fitted with plastic cannulas. The cannulas were built with a threaded cap that could be readily removed, giving access through a 4-in. opening into the rumen. The animals were maintained on 4 lb. of grain per day with hay and water ad libitum. All feed was removed from the animals after the evening meal the night before each experiment.

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In Experiment 1, six trials were made, samples of top and bottom ingesta being taken at hourly intervals beginning just before the morning feeding. The duration of each trial is indicated in Table 1. In Trial 1, animal A was fed 2 lb. of grain plus all the hay it would consume in 1 hour. In Trial 2, with the same animal, 40 g. of urea was added to the grain. In Trials 3 through 6, the feed was the same as in Trial 1, but the animals were fed again 8 hours after the morning feeding. In Trials 3 and 4, animal B was used on different days. In Trials 5 and 6, animals B and C, respectively, were used on the same day.

Samples of top ingesta were taken by hand approximately 15 in. anterior to the fistula by selecting material from about 3 in. below the top of the hay "mat." Bottom ingesta were collected by covering the top of a 1-pt. wide-mouth bottle with the palm of the hand while the bottle was lowered to the floor of the rumen. There it was opened and allowed to fill spontaneously. When full, the bottle was closed with the hand again while it was removed from the rumen. Samples of top, middle, and bottom ingesta were taken in Trials 1 and 2, but only top and bottom samples were taken in Trials 3 through 6.

Analyses of rumen ingesta. At the time of sampling, the sub-samples for proximate analyses were placed in pint cartons and refrigerated until analyzed by standard procedures. Sub-samples for analysis of soluble components were obtained by squeezing the ingesta through two layers of cheesecloth. The juice was then appropriately diluted for analysis. Volatile fatty acid analyses were made according to the method of Neish *(11)*, as modified by Bauman *(2)*, Lengemann *(7)*, and Moore *(10)*.

Water-soluble sugars, nonprotein nitrogen, and ammonia nitrogen determinations were made by using aliquots from a 1:10 dilution of the rumen juice in 11% trichloroacetic acid. Alcohol-soluble sugars were determined by using aliquots from a 1:10 dilution of rumen juice in 95% ethanol. Total nitrogen in the juice was determined by using an aliquot from 1:50 dilution of the juice in water.

Ammonia concentrations were determined on aliquots from the clear supernatant obtained by centrifuging the trichloroacetic acid extracts. One ml. of the extract was mixed in an 18 × 150 mm. test tube with 2 ml. of water, 2 ml. of Nessler's reagent (1.75 g. of gum ghatti plus 7 g. of K_2HgI_4 dissolved in 800 ml. of water by heating under reflux, filtering, and diluting to 1 l.), and 3 ml. of 3.3 *N* NaOH. The optical density at 480 mμ was read after 15 minutes. The amount of ammonia in the sample was then determined by reference to a standard curve prepared with each set of samples by using recrystallized $(NH_4)_2SO_4$ as the standard. This procedure has been found to give essentially quantitative results by recovery of known amounts of ammonia from rumen fluid and by comparison with results obtained by titrimetric determination of the ammonia in rumen samples after distillation of the ammonia into boric acid from $Ca(OH)_2$.

Nonprotein nitrogen was determined by micro-Kjeldahl analysis of 0.5-ml. aliquots of the trichloroacetic acid extracts according to the method of Johnson *(6)*. This method actually measures the sum of ammonia nitrogen and organic nitrogen in the form of amino acids and other nitrogenous organic compounds of molecular weight too small to be precipitated by trichloroacetic acid. Organic

TABLE 1

Stratification in the concentration of ammonia (γN/ml) in rumen ingesta of fistulated steers as a function time since feeding (Experiment 1)

Hours since feeding	Trial 1			Trial 2			Hours since feeding	Trial 3		Trial 4		Trial 5		Trial 6	
	Top	Middle	Bottom	Top	Middle	Bottom		Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
0	220	185	145	180	170	135	0	296	97	275	220	276	190	250	250
1	140	150	185	580	470	305	1	150	56	335	355	390	360	330	410
2	230	220	180	415	460	415	2	271	243	355	305	415	345	440	390
3	290	225	190	435	480	325	3	268	135	355	290	390	355	405	285
4	250	205	165	385	300	260	4	236	177	290	230	335	330	330	210
5	210	170	165	310	235	210	5	96	57	240	205	310	196	200	145
6	180	175	165	250	205	195	6	110	65	170	175	—	150	170	90
7	150	145	135	200	135	130	7	108	132	175	168	223	116	133	110
8	125	130	115	115	135	145	8	105	105	188	170	220	115	120	98
9	200	140	120	160	150	145	1	116	203	255	275	350	305	260	303
10	145	125	111	165	170	160	2	183	234	333	285	390	345	370	350
11	235	160	115	210	155	140	3	157	211	318	280	400	285	440	290
12	300	200	200	150	120	120	4	257	174	280	238	310	260	420	330
13	225	225	225	120	125	105	5	176	113	245	210	—	—	—	—
14	—	—	—	95	115	120	6	—	—	—	—	—	—	—	—

nonprotein nitrogen was calculated by subtracting ammonia nitrogen from nonprotein nitrogen. Total nitrogen in the strained rumen juice was determined by the micro-Kjeldahl procedure cited above by using 1-ml. aliquots of the aqueous dilutions.

Water-soluble sugars were estimated in terms of glucose equivalents by colorimetric analysis of trichloroacetic acid extracts. A known volume of extract was diluted to 4 ml. with distilled water in an 18 × 150 mm. Pyrex test tube. Eight ml. of anthrone reagent (2 g. of anthrone dissolved in 950 ml. of conc. H_2SO_4 plus 50 ml. of water) was then added rapidly, and the contents were mixed immediately. The optical density at 630 mμ 15 minutes after mixing was determined, and the amount of water-soluble carbohydrate in the samples was determined from a standard curve of glucose analyzed simultaneously. This analysis estimates not only glucose but also other hexoses, pentoses, and the higher oligosaccharides that have become dissolved in the ingesta. The absolute values in terms of glucose are without meaning, since the color yield with anthrone depends upon the particular sugar tested. On the other hand, the determination does give a fairly clear estimate of the relative amount of sugars in the various samples, if it is assumed that the distribution of sugars is fairly uniform between samples.

Alcohol-soluble sugars were determined on the ethanol extracts with the same anthrone analysis as that described above. These data represent the relative concentrations of low molecular weight sugars, such as pentoses and hexoses, in the ingesta, since starch and the dextrins of reasonably large molecular weight are insoluble in alcohol.

Proximate analyses were made by standard procedures, but in Trials 5 and 6 all samples of bottom ingesta were centrifuged until a clear supernatant was obtained. The sediment was then used in further analysis. Top samples were analyzed as taken from the animal. As a result of the centrifugation most of the dissolved compounds in the bottom ingesta were lost, including ammonia, sugars, amino acids, and dissolved salts. Consequently, the N.F.E. and protein data are of little significance and have been omitted from the tables and graphs. All proximate analyses were calculated to an ash-free dry matter basis because of heavy and variable contamination of the bottom samples with sand from the floor of the rumen. In Experiment 2 (the rumenotomy) proximate analyses were carried out on the samples as taken from the animals without centrifugation so that comparisons of top and bottom samples would be valid.

The ability of ingesta samples to digest cellulose was estimated by the method proposed by Henderson *et al. (5)*, modified as follows: A 500-mg. square of Patapar (Paterson Parchment Paper Company, Bristol, Pa.) weighed to the nearest 0.1 mg. was placed in an 18 × 150 mm. test tube and weighted with a piece of wire. After the tube had been nearly filled with strained rumen juice, a layer of mineral oil about ¼ in. thick was added to the top to insure anaerobiosis. The tube was placed in a 39° C. incubator. After incubation for 24 hours the paper was removed from the tube, washed free of plant debris with a gentle stream of water, dried, and weighed again. The per cent digestion was then

calculated. Results obtained by this analysis are dependent upon pH, buffering capacity, and other properties of the samples, as discussed later.

The pH of ingesta samples was determined with a glass electrode immediately after the sample had been removed from the animal. Titratable alkalinities to pH 5.0 and 4.0 were then determined, with the same 25-ml. aliquots, with 0.10 N H_2SO_4.

Stratification of the ingesta from a normal animal. In Experiment 2 a rumenotomy was performed on a mature Holstein cow in late lactation for the purpose of establishing whether the stratification which had been consistently observed in the fistulated steers was an artifact resulting from impaired rumen motility as a result of the cannula. The animal was fed 4 lb. of grain at 8:00 A.M. and hay ad libitum in a paddock from 8:30 A.M. until 10:30 A.M. on the day of the experiment and was then placed in a stanchion. At 1:30 P.M. she was fed 3 lb. of grain and while eating was anesthetized locally by a modified paravertebral nerve block with 2% procaine. The incision was made in the usual left paralumbar region. Anesthesia was complete, and the animal showed no signs of discomfort during the operation. Rumen motility was apparent throughout the operation. Top ingesta were taken from about 12 in. anterior to the incision. When 2 qt. had been collected and mixed, two 1-pt. sub-samples were taken for proximate analysis. The remainder of the sample was then strained through two layers of cheese cloth. While one man was determining the pH and titratable alkalinities, another prepared the dilutions needed for the remainder of the analyses. A third man began collecting the second sample of top ingesta from a position as close as possible to the place where the first sample was collected. Duplicate bottom samples were then taken as described for fistulated animals and processed in the same manner as the top samples.

RESULTS AND DISCUSSION

Stratification and kinetic changes. Inspection of the data presented in Table 1 indicates that the range in ammonia concentration and the magnitude of the changes after feeding are similar to those reported by Annison *et al. (1)* and el-Shazly *(3, 4)*. Volatile fatty acid data (Table 2 and Figure 1) are comparable to those of el-Shazly *(4)*.

From Figure 1 it can be seen that there are differences in composition of the top and bottom ingesta. Top ingesta were characteristically higher in total nitrogen, nonprotein nitrogen, organic nonprotein nitrogen, ammonia, water- and alcohol-soluble sugars, volatile fatty acids, and crude fiber, but lower in ether extract, pH, titratable alkalinity to pH 5.0, and per cent digestion of added cellulose in vitro. The statistical analysis summarized in Table 4 shows significant or highly significant differences in composition between top and bottom ingesta, with the exception of the alcohol-soluble sugars (where significance is approached) and titratable alkalinity to pH 4.0.

Undoubtedly, rumination and consequent exposure of fresh substrate to the microflora contributed to the observed variability. In Trials 5 and 6, the animals consumed more hay during the second feeding; as indicated in Figure 1, the

TABLE 2

Variation between samples and locations in the composition of rumen ingesta from a normal cow three hours after feeding (Experiment 2)

	Rumenotomy samples	pH	pH 5 titr. alkalinity	pH 4 titr. alkalinity	Water-sol. sugar	Alcohol-sol. sugar	Total N	NH_3-N	N.P.N.[a]	Cellulolysis	Butyric	Propionic	Acetic	E.E.[b] (ash free)	C.F.[c] (ash free)
			(meq/25 ml.)		———		*(γ/ml)*	———		*(%)*	——*(meq/100 ml.)*——			*(%)*	*(%)*
Top															
(av. of duplicates)	1	6.09	1.09	3.01	845	612	3,080	248	901	5.8	4.00	3.86	13.26	2.77	30.9
	2	5.89	1.14	3.40	735	672	3,050	249	860	6.6	3.84	3.85	13.65	2.82	30.5
Bottom															
(av. of duplicates)	1	6.54	1.60	3.16	350	470	1,305	184	473	24.9	2.48	2.70	9.73	3.27	21.0
	2	6.51	1.62	3.28	415	490	1,358	185	474	26.5	2.32	2.46	9.45	3.01	17.3

[a] N.P.N. = nonprotein nitrogen.
[b] E.E. = ether extract.
[c] C.F. = crude fiber.

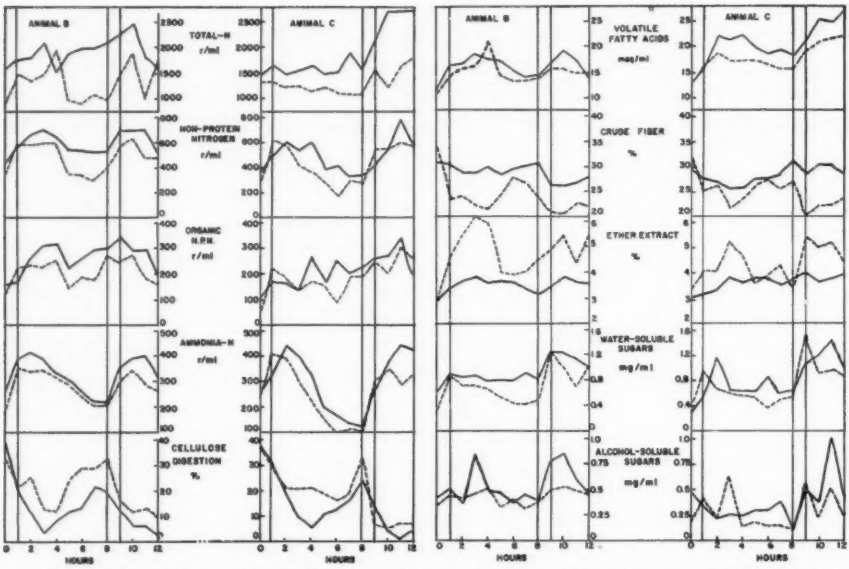

FIG. 1. Hourly fluctuations and stratification in the composition of rumen ingesta of two fistulated steers. The animals were fed during the intervals: 0-1 hour and 8-9 hours. Solid lines represent top ingesta and dotted lines represent bottom ingesta.

concentrations of the ingesta constituents were affected by this and the shorter feed interval.

This stratification is apparently a general characteristic of rumen ingesta, for it was also observed in approximately the same magnitude in the older dairy animal when samples were obtained at the time of rumenotomy (Table 2). The rumenotomy data establish that the stratification is not an artificial condition induced by fistulation.

Statistical interpretations. The statistical analysis in Table 3 shows that the ammonia concentration of rumen ingesta varies more between days in the same animal than between animals on the same day despite the fact that the rations in all trials were similar. Tables 3 and 4 show that there was generally a highly significant difference in the variables measured between hours as related to feeding time. This means that the fluctuations over a period of time (Figure 1) are real, although the shape of the curves may not be exact. Consequently when samples are desired that are independent of an animal-by-time interaction, they should be taken on the basis of time since feeding rather than chronological time. This caution has been expressed by others but needs to be tempered by the observation of a significant animal-by-time interaction for the nonprotein nitrogen and volatile fatty acids and an interaction for the cellulolytic activity that approached significance. The interaction may be a reflection of subtle differences in microflora or absorption rates but indicates that sampling in relation to feeding time will not remove all of the error. The absence of interaction for the other variables

TABLE 3
Statistical summary evaluating the effect of day, time of day, ration, and sampling location on the ammonia concentration of rumen ingesta from fistulated steers

	Trials 1 and 2		Trials 3 and 4	
	D.F.[a]	NH_3-N/ml	D.F.	NH_3-N/ml
Between days—same animals				
Different ration	1	**[c]	—	—
Same ration	—[b]	—	1	**
Between hours	8	**	13	**
Between positions	2	N.S.[d]	1	**
Interactions:				
Day × hours	8	N.S.	13	N.S.a[e]
Day × positions	2	N.S.	1	N.S.
Hours × positions	16	N.S.	13	N.S.

[a] D.F. = degrees of freedom.
[b] — = no observed data.
[c] ** = significant at $P < 0.01$.
[d] N.S. = not significant.
[e] N.S.a = approaches 0.05 but not significant.

between animals and time demonstrates that similar changes in rumen ingesta take place in relation to time in each of the animals tested.

In Trials 1 and 2, in which samples were taken from top, middle, and bottom ingesta, the differences in ammonia concentration were not great enough to be statistically significant, even though the ammonia concentration of samples from the middle portion generally fell intermediate between that of top and bottom samples. The raw data appear in Table 1, and the statistical analysis is summarized in Table 3. Certainly, one difficulty in this experiment was the selection from all three regions of samples that were uncontaminated with material from adjacent locations.

When water-soluble sugars are considered, the positions (top and bottom) react differently with respect to time. This demonstrates that the cross-overs in concentration in top and bottom samples (Figure 1) were real. It indicates distinct physical pathways and localized concentrations, which maintain their identity throughout most or all of the day. A significant interaction between hours and position was approached by ether extract and cellulolytic activity, as measured by this procedure. Ether extract was the only variable measured that showed significant animal-by-position interaction; therefore, the ether extract was distributed differently in the top and bottom ingesta of the animals studied.

The statistical evidence is not entirely additive for stratification or time relationships since many of the variables measured are physiologically related, either directly or indirectly. For example, as shown in Table 4, ammonia, nonprotein nitrogen, and organic nonprotein nitrogen all varied significantly with time. Here the ammonia was actually a fraction included in the nonprotein nitrogen. In contrast, the concentration of total nitrogen in the juice from the ingesta did not vary significantly from hour to hour, but the form in which the nitrogen occurred was variable. These evidences are additive.

The data represented in Table 4 are from only two animals. Since the stratification and hourly variation show significance in most instances, the differences

TABLE 4

Statistical summary evaluating the effect of animal, time of day, and sampling location on the composition of rumen ingesta of fistulated steers (Trials 5 and 6)

	D.F.[a]	NH_3-N/ml	Total N/ml	N.P.N./ml	Ether extract (ash-free)	Crude fiber (ash-free)	D.F.	Total vol. fatty acid	D.F.	Alcohol-sol. sugar/ml	Total sugar/ml	Cellulolysis
								(meq/100 ml.)				*(%)*
Between animals same day	1	N.S.[b]	N.S.	**[c]	N.S.	N.S.	1	**	1	**	N.S.	N.S.
Between hours	11	**	N.S.a[d] (2.6/2.82)	**	**	N.S.	10	**	12	N.S.a (2.62/2.69)	**	**
Between positions	1	**	**	**	**	**	1	**	1	N.S.a (4.44/4.75)	**	**
Interactions:												
Animal × hours	11	N.S.	N.S.	*	N.S.	N.S.	10	*	12	N.S.	N.S.	N.S.a (2.29/2.69)
Animal × position	1	N.S.	N.S.	N.S.	*	N.S.	1	N.S.a (2.38/2.97)	1	N.S.	N.S.	N.S.
Hours × position	11	N.S.	N.S.	N.S.	N.S.a (2.44/2.82)	N.S.	10	N.S.	12	N.S.	*	N.S.a (2.67/2.69)

[a] D.F. = degrees of freedom.
[b] N.S. = not significant.
[c] ** = significant at $P < 0.01$.
[d] N.S.a = approaches 0.05 but not significant. Figures in parentheses are $F_{obs}/F_{0.05}$ where significance is approached.
[e] * = significant at $P < 0.05$.

must be either relatively large or very consistent. Statistical analysis of the rumenotomy data presented in Table 2 reveals significant or highly significant differences between positions (top and bottom) for all variables measured except titratable alkalinity to pH 4. With this number of observations, only pH showed significant difference between samples from the same location.

Physiological significance. Aside from the statistical differences that occur in the composition of ingesta from the top and bottom of the organ, there is the remaining question of whether these differences are large enough to interpret physiologically. The values reported on the graphs in Figure 1 indicate that the top and bottom of the rumen do provide a sufficiently different environment to affect to a considerable degree the rate, and possibly the course, of the fermentation.

All of the data are compatible with the hypothesis that the physical pathways of hay and grain particles through the rumen are very different. Visual examination of rumen ingesta for a few hours after feeding hay and grain at the same time also suggests that the two feed components move independently. The coarse hay particles collect in a "mat" in the top of the rumen and remain there for many hours. In contrast, grain particles are initially mixed throughout the rumen ingesta after eating, but they rapidly settle to the floor of the rumen and reticulum and then pass on to the omasum. In the "mat" the chemical components of the hay are digested in order of decreasing solubility and digestibility, the readily fermentable fractions being destroyed first. As a result of the gasses produced during this initial fermentation of the hay, particles that settle to the floor of the rumen are buoyed back up into the "mat." Consequently, even when the top ingesta are tightly packed and very dense and dry, a considerable volume of very liquid ingesta is present in the bottom of the rumen. Hay particles, then, remain in the top of the organ for the most part until, through chewing and fermentation, they have lost their particulate identity and are no longer large enough to be carried back to the top by gas bubbles.

If this outline of rumen function approaches a valid description of the behavior of hay and grain particles in the rumen, the stratifications and time changes shown in Figure 1 gain increased significance physiologically. For example, it would be anticipated that the fermentation intermediates and products associated with active fermentation of hay would tend to attain higher equilibrium concentrations in the top ingesta than in the bottom when a low-grain ration such as that fed here was consumed. The enhanced volatile fatty acid, ammonia, and free sugar levels in the top suggest that this was the case. The higher nitrogen values from top ingesta suggest the possibility that the concentration of microorganisms may also be greater in this nutritionally superior environment.

Higher crude fiber in the top for several hours after feeding again indicates a localized concentration of the hay particles there. In addition, the crude fiber level in the ingesta can be seen to have crossed over during the 15-hour fast that preceded the experiment so that the samples taken before the morning feeding show a higher crude fiber in the bottom. This cross-over presumably reflects the

accumulation in the bottom of hay particles from which most of the readily fermentable components have been removed. The rate of microbial attack on the residuum has decreased to the point where bubble formation on the particle surface no longer supports it in the "mat."

Preliminary gravimetric analyses of digestion of cellulose samples in vivo indicate that the cross-over of crude fiber content in top and bottom samples represents the settling of roughage material ingested 36 to 48 hours previously.

Water-soluble sugars are generally higher in the upper ingesta because they are being liberated from the roughage there. Just after feeding, however, the soluble sugars in the grain and those freed by amylase action increase the sugar level in the bottom ingesta, since the grain quickly settles out of the hay "mat." There is a similar transitory rise in the ether extract of bottom samples after feeding. This pattern is not as clear in the alcohol-soluble sugars.

By the inspection of the curves in Figure 1, crude fiber appears to be positively associated with total nitrogen, ammonia, nonprotein nitrogen, water-soluble sugars, and alcohol-soluble sugars and negatively associated with ether extract and in vitro cellulose digestion. Ether extract and in vitro cellulose digestion were positively associated, but this may reflect only the difference in pathways of hay and grain rather than a direct metabolic relationship.

The more rapid in vitro digestion of added cellulose by bottom samples of ingesta appears to be caused by the higher initial pH and buffering capacity and by the lower concentration of competing substrates. Attempts to clarify these relationships are currently in progress. The apparent independence of titratable alkalinity to pH 4 with respect to initial pH and the titratable alkalinity to pH 5 may indicate that at least two buffering systems are active in the rumen over this range. There was a noticeable increase in buffering activity at approximately pH 5, as previously observed by Turner and Hodgetts *(15)*. Differences in pH and buffering capacity similar to those reported here have been observed by Smith *(14)* and Turner and Hodgetts, although the latter did not interpret the differences to be real.

SUMMARY

Samples of ingesta from the top and bottom of the rumen of three 2-year-old fistulated steers were taken at hourly intervals over a 12-hour period that included two feedings. The samples were analyzed for crude fiber, ash, ether extract, total nitrogen, ammonia, nonprotein nitrogen, water-soluble sugars, alcohol-soluble sugars, volatile fatty acids, and the digestion of cellulose in vitro. In all instances except alcohol-soluble sugars, significant or highly significant differences were observed between the top and bottom samples. Concentrations of all factors measured except ether-extract and the amount of cellulose digestion in vitro were significantly higher in top samples. These data together with the significant time fluctuations indicate that hay and grain particles follow distinctly different physical pathways in traversing the rumen. In a control experiment, duplicate samples were taken from both the top and the bottom of the rumen of a normal (unfistulated) mature Holstein cow by rumenotomy 3 hours

after the morning feeding. These data show the same stratification as that seen in the samples from the steers. In additional analyses, pH and titratable alkalinity to pH 5.0 were significantly higher in bottom than in top samples. Observation of stratification in the rumen of the normal mature cow is evidence that the phenomenon is not an artifact resulting from fistulation.

ACKNOWLEDGMENTS

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TECHNICAL NOTES

THE EFFECT OF THYROIDECTOMY AND IODINE SUPPLEMENTATION ON THE PLASMA PBI OF A JERSEY BULL

The adoption of the plasma level of protein-bound iodine as an index of thyroid activity in dairy cattle has been hampered by uncertainty as to the proportion of the PBI which is thyroxine and the closeness with which changes in PBI reflect variations in the thyroid secretion rate.

A recent investigation of the effect of thyroidectomy on the PBI of a Jersey bull has shed some light upon these questions. The bull was thyroidectomized at 8 months of age by the subcutaneous administration of 30 mc. of carrier-free I^{131}. Within 18 days the PBI had dropped to 3.4 γ % from the pretreatment level of 6.7 γ % (Figure 1) and on the 33rd day the concentration was 0.8 γ %. During the next several months the PBI level ranged between 1.4 and 0.0 γ % with an average of 0.5 γ %.

These results suggest that the PBI of dairy cattle is closely related to thyroxine although Reece and Man *(2)* found that the butanol extractable iodine accounted for only 57.2% of

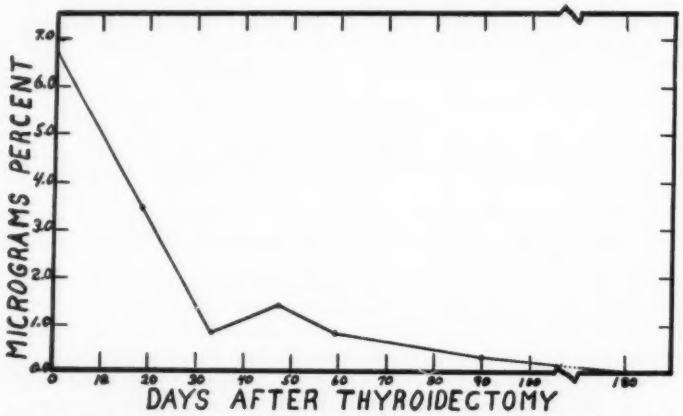

FIG. 1. The effect of thyroidectomy with I^{131} upon the plasma PBI concentration of a Jersey bull.

bovine sera PBI. Although the apparent discrepancy between these data may be due to differences in the iodine content of the rations used, the calves in the present study received

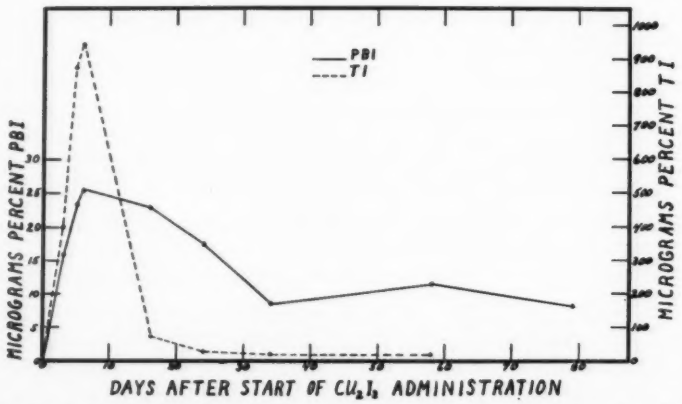

FIG. 2. The effect of oral administration of cuprous iodide upon the plasma total and protein bound iodine of a thyroidectomized Jersey bull.

no supplemental iodine. Long *et al. (1)* have shown that up to 22 mg. of supplemental iodine daily produced no measurable effect on the PBI concentration of cows.

In order to learn if dietary iodine could affect the PBI concentration of the thyroidectomized bull, six consecutive daily oral doses of 1 g. of cuprous iodide (66.7% iodine) were administered. This large dosage caused a major increase in the plasma PBI as well as in the total iodine (Figure 2). The concentrations rose from 0.0 γ % PBI and 1.8 γ % TI before treatment to a high of 25.5 and 948 γ %, respectively, on the last day of treatment. Repeated washings with distilled water failed to remove this PBI fraction.

Since the TI level was 20.2 γ % 52 days later and the PBI was 8.4 γ % 73 days after the cessation of supplementation, it appears that large doses of iodine may continue to influence the plasma iodine level for a considerable length of time. Whether smaller amounts of iodine would have produced similar results except for magnitude is conjectural. One may speculate, however, that the plasma PBI of dairy cattle receiving supplemental iodine will contain a nonthyroidal iodine fraction even though, at the usual levels of feeding, present analytical techniques may not show significant differences between supplemented and unsupplemented animals.

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THE PRESENCE OF α-KETOGLUTARIC ACID IN MILK[1]

During a study of the acids generated by heating milk *(3)*, a compound behaving in the manner of α-ketoglutaric acid (KGA) was observed. The presence of this compound in unheated controls also was noted. A reasonably careful search of the literature revealed no reports concerning the presence of KGA in milk; thus, the following effort was made to render the identification conclusive.

The methods of Cavallini and Frontali *(2)* were used to convert the keto acids in 10 ml. of pasteurized skimmilk to their 2,4-dinitrophenyl (DNP) hydrazones and to chromatograph the latter. The dominant spot which developed under these conditions coincided closely in R_f value with that from the authentic derivative of KGA.[2] The keto acid responsible for the spot from milk was isolated as its DNP hydrazone from 500 ml. of pasteurized skimmilk, the reagents and procedure *(2)* being adapted to the larger sample. The resulting ammoniacal solution of the keto acid derivative was acidified and extracted with ether, and the ether was evaporated to gain the crude product. Impurities were removed from this material by extraction several times with small quantities of boiling hexane. Three recrystallizations of the hexane-insoluble material from a mixture of ethyl and petroleum ether gave a bright yellow microcrystallin product in a yield of about 4 mg. This compound showed the following properties in common with the authentic KGA DNP hydrazone: R_f value of 0.26 by the paper chromatographic method of Cavallini and Frontali *(2)*, absorption maximum of 382 mμ in phosphate buffer (pH 7.2), m.p. 215° to 200° C. (decomp.). Mixing the known and unknown together did not depress this decomposition point, although mixing the DNP hydrazone of pyruvic acid (m.p. 220° C.), with the derivative of KGA depressed the decomposition point to 195° C.

As a control measure for the DNP hydrazone aspect of the isolation procedure, direct demonstration of KGA in the tungstic acid filtrate from milk was attempted. The filtrate from 50 ml. of pasteurized skimmilk was exhaustively extracted with ether, the ether was removed on a steam bath, and the residue was mixed with 0.1 ml. of water. This mixture was chromatographed on paper by the method of Buch *et al. (1)* by using their pentanol-formic acid solvent system and bromophenol blue as the spray reagent. This chromatogram revealed a spot (Rf 0.47) originating from the milk extract, the position of which coincided with that from authentic KGA. Three additional spots on this chromatogram from the milk extract were observed to correspond with citric, lactic (and/or pyruvic), and hippuric acids.

It was considered of interest to know approximately what levels of KGA might be met in milk. By using the quantitative method of Cavallini and Frontali with minor modifications, duplicate values of 14.8 and 15.4 mg. per liter were obtained for one sample of pasteurized skimmilk. One week later, a second sample from the same milk supply gave duplicate values of 13.4 and 15.5 mg. per liter. Two

[1] Authorized for publication 3/6/56, 1956, as Paper No. 2047 in the Journal Series of the Pennsylvania Agricultural Experiment Station.
[2] Faint spots moving in the manner of pyruvic and α-keto isocaproic acids also were observed.

plausible sources of KGA in milk are: residual from the synthesis of glutamic acid for certain of the milk proteins and by filtration from the cow's blood.

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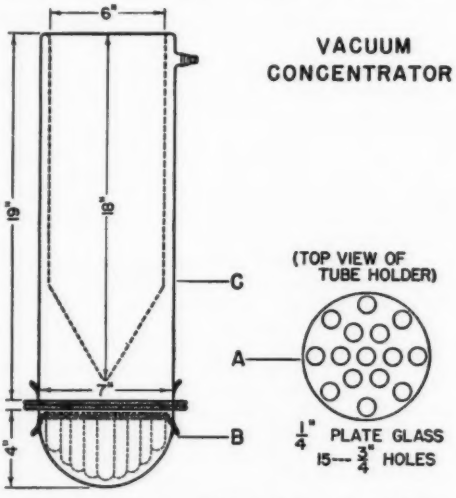
A NOVEL GLASS VACUUM CONCENTRATOR

An all-glass vacuum concentrator has been developed which is highly efficient in drying a variety of biological materials from the frozen state. A novel feature of the concentrator is the method of sealing the evaporating surface to the cold, condensing surface. The seal is made by two ground glass surfaces in proximity as in a desiccator and top. A short, unobstructed vapor path for maximum efficiency is possible with this design. The equipment is easily assembled and disassembled, permitting ready access to the dried sample, and can be conveniently adapted to either small or large scale concentrations.

This concentrator has been employed effectively in removing water from various biological preparations, including bacteria. It has been used for low temperature removal of formic and acetic acids and ammonium formate. The evaporation of 500 ml. of water can be accomplished in 8 to 10 hours when the water is frozen directly on the evaporating surface. For small scale evaporations, a glass plate *(A)* adapted to carry test tubes can be inserted into area *(B)*, as shown in the diagram. The evaporation rate from test tubes varies from a few hours up to 12 hours, depending upon volume and supplementary heating. As insulation is provided by the vacuum surrounding the evaporating surface, heating greatly improves the efficiency when the test tube adaptation is used. Overnight operation readily assures evaporation of 6 to 10 ml. with no heating.

When either alcohol or acetone with dry ice is used as a cooling medium, the inside chamber has sufficient capacity to hold up to 15 hours without attention. Insulation of the cooling chamber *(C)* from the top down to the spring hooks has been found to increase the holding time.

The large size shown in the accompanying figure is one useful form of the design. A smaller model with a diameter of 4 in. and an over-all length of 20 in. is also being used and is equally effective when dry ice can be added at about 6-hour intervals. A vacuum



pump of 10 l. per minute free air displacement is adequate to operate two concentrators simultaneously. A trap containing a mixture of sodium hydroxide pellets and calcium chloride is routinely used as a safety precaution to protect the pump when water and formic acid solutions are concentrated.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Have We Properly Emphasized Flavor In the Quality Yardstick?

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Marked improvements have been made in the general quality of milk and its derivatives during the past quarter-century. The chief quality improvements have been in the esthetics of milk production, in sanitation, and in reduced bacteria counts. Flavor has been considered, but usually defensively. Good flavor has been taken for granted by many. Why not? After all, does not the cow give good-flavored milk? Has not our responsibility for flavor control ended when we have safeguarded the milk against bacterial spoilage? Unfortunately, this trend of thinking has prevailed too long. Flavor in the quality yardstick needs reemphasis.

It is doubtful if the dairy industry yet is fully aware of the hidden potentialities of flavor in selling dairy products. Competitive food industries frequently stress the flavor of their products and place less emphasis on the nutritive values. Perhaps the dairy industry could learn some lessons from this approach and at the same time consider the very essential part which flavor possesses as a measure of quality.

Food Industry Becoming Flavor-Minded

The entire food industry today is so conscious of the importance of flavor that research workers frequently make use of taste panels in their attempt to maintain flavor standards and to achieve superiority of flavor. At the 1955 meeting of the Institute of Food Technologists no less than seven papers related to flavor, wholly or in part. That the dairy research worker is becoming more and more aware of the important role that flavor plays in the sale of dairy products is shown by the nature of the research papers presented at the annual meetings of the American Dairy Science Association. At the 1930 meeting only two papers related to flavors, whereas at the 1955 meeting there were nine. Probably more research has been done and more articles published on one single group of flavors—the oxidized flavors of milk—during the last quarter-century than on any other single subject in the field of dairy manufacturing.

Flavor a Significant Factor in Milk Consumption

The important role that flavor plays in milk consumption is not fully appreciated. Most people, especially the young, drink milk because they like it. Parents admonish their children to drink milk for reasons of health, growth, bone structure, sound teeth, etc. Perhaps in habit-forming years many youngsters drink milk under duress, but when they are older they will continue to drink milk only if it gives them gustatory satisfaction and not because it is "good for them." Little does the average youngster concern himself with the problems of health, crooked bones, or loss of teeth. The average American child believes that, barring the hazards of childhood, he has several years' mortgage on good health, and it is not until his good health begins to wane that he becomes concerned. Heretofore, he has accepted this blessed gift as though it were his inherently and not as a loan in trust to be protected and carefully guarded.

Improved facilities for the production, handling, and transporting of milk to the plant and better controlled methods of processing make possible the marketing of milk and milk products of excellent flavor. Under proper storage these items have a much longer shelf life than did similar products a few years ago. Under such conditions an ample supply of good-tasting milk, cream, cheese, and other milk products can be kept in the home at all times. It is not uncommon for the housewife to store milk and cream, for example, for 1-2 weeks without danger of spoilage or flavor deterioration.

Research Has Corrected Certain Misconceptions Regarding Milk Flavors

The prevailing concept of the quality of dairy products at the turn of the century to a large extent involved richness, as measured by the percentage of fat. Little was recorded in the literature concerning the flavor of the milk, except for the taints produced by, or attributed

to, bacterial growth. Interestingly, most off-flavors, if not due, or alleged to be due, directly to bacteria, were thought to be normal for the season, the cow, or the stage of lactation; thus, it was believed that little could be done about them. Apparently, much ignorance prevailed about the off-flavors of milk. Like "old wives' tales," circumstantial evidence was passed on, and this misinformation stuck. Indeed, it was difficult to believe the truth, once the truth was revealed by scientific investigation.

During this early period, the dairy industry seemed to lack faith in the consumer's ability to taste and smell. Considerable reliance was placed on chemical tests in diagnosing flavor troubles. Actually, the average person's ability to taste and smell is phenomenal. Consider a chemical test that can detect and identify with assurance in a fraction of a second, or in a few seconds at most, the presence of a trace of a substance in the concentration of one part in a billion, or 0.0000001%. Such a tool is the ever-present, readily available, safely guarded olfactory sense with which every normal man is endowed. Bernard *(1)* well states:

> "Your nose can receive, analyze and sort out odors with a speed and finesse that no laboratory instrument can duplicate. When you notice the perfume of a girl you pass on the sidewalk, you are sensing an amount of odor so infinitesimal that no mechanical device now known could either detect or record it. While it's true that a bloodhound can follow a spoor better than you can (partly because his nose is closer to the ground), your human sense of smell is definitely no slouch. At top efficiency, your nose can probably detect as little as two-trillionths of a gram of a strong-smelling chemical."

Virtually no appreciation was given by the early dairy processor to the application of knowledge about the physiology or psychology of the chemical senses—taste and smell. The lack of scientific data added to and/or perpetuated this era of ignorance about off-flavors in milk and their causes. Now we shudder at the early prevailing, nonquestioned veterinarian's recommended dosage of 1 lb. of Epsom salts to cure a cow giving "strong" (rancid) milk. Likewise, we cringe when it is recalled that the early complaints on the "cardboard" (oxidized) flavor were explained away as being due to ice-box exposure to meat, particularly bacon, or to poor paraffining of the bottle cap. Happily, recent research on food flavors has made possible more accurate diagnosis of flavor defects. Today the experienced dairy flavor specialist can spot the chief contributing cause of an off-flavored milk with the same assurance as the experienced country doctor who can diagnose a case as scarlet fever immediately after whiffing the air of the sick-room.

Some Developments Which Led Industry to Evaluate Flavor

A number of technological developments have caused the dairy industry to become more flavor-conscious. The more important innovations were: improvements in detergents and sanitizers; rural electrification; mechanical refrigeration; good roads, motor transportation, and mobile refrigeration; antibiotics; more rigid inspection; less frequent home delivery; homogenization; bulk handling; pipe-line milking; 5- or 6-day plant operation; single service containers; high-temperature, short-time pasteurization; metal alloys; and the shift to store sales.

Three major results have accompanied these developments, namely, *(a)* lower bacterial counts; *(b)* wider outer-market distribution of milk; and *(c)* longer storage of milk before consumption. Thus, the flavor of the milk must be clean and sweet at the time of packaging and must remain so over an extended period.

Impact of Technological Progress on Quality of Product

The results of research have forced the flavor specialist to change his concept of quality. He has discovered new causes of off-flavors. Markedly lower bacterial counts are found in the raw milk supply. Furthermore, the growth of both the bacteria surviving pasteurization and those entering the product through post-pasteurization contamination is inhibited by low-temperature storage. Consequently, a new type of flavor spoilage occurs. This is of a chemical rather than a bacteriological nature. Today, bacteria play a minor role in present-day flavor changes in pasteurized milk stored at low temperatures. In fact, lack of bacterial metabolism releases the oxygen in the milk for chemical union with the fatty-constituents, thus resulting in oxidized flavors.

More Emphasis Should Be Placed on Flavor

The dairy industry must pay more attention to the flavor of its products in order to protect itself against the potential competition of other foods. The high level of annual milk consumption (385 lb. per capita) in the United States seems phenomenal when one considers that the consumer is confronted with innumerable varieties of food at the supermarkets in every sizable city throughout the country. There must be some basic reasons for the relatively high level of milk consumption by 160,000,000 people of the United States, who have the opportunity to buy so many beguiling foods. It is believed that in spite of its known nutritional value, its availability, its relative cheapness, and the influence of constant advertising, promotional schemes, and education in the schools, people drink milk primarily because they like it and because they have the assurance that it is free from disease germs and has been produced and handled in a hygienic manner.

Although the United States does not rank the highest among the nations in total milk

consumption (eighth), no country surpasses it in the volume of beverage milk consumed. This country has attained this enviable position, undoubtedly, because the flavor of the milk has been in keeping with the nutritional qualities it is known to have. A traveler may order a glass of milk in Minneapolis, Hartford, or Los Angeles with reasonably good assurance of getting a highly palatable, wholesome product.

The milk industry not only must guard the flavor of its product if it is to continue to meet competition from without, but it must be alert to the increasing competition from within the industry itself. Beverage milk is a good source of milk protein. However, nonfat dry milk, another good source of proteins, has entered the competition beyond the most fanciful dreams of its distributors (Table 1). The

TABLE 1

The per capita consumption of cheese and nonfat dry milk in the United States[a]

(*USDA, Agr. Sta.*, 1954. p. 393)

Year	Cheese	Nonfat dry milk
	(lb.)	*(lb.)*
1940	5.9	2.2
1945	6.6	1.9
1950	7.6	3.6
1953[b]	7.1	4.0

[a] Included are all kinds of cheese except cottage, pot, bakers', and full-skim American.
[b] Preliminary report.

recent "instantizing" of the dispersion of this improved product in water has removed an important barrier to its ready acceptance by the housewife. The sales of packaged nonfat dry milk within the last 10 years have been phenomenal.

In addition, cheese is becoming more and more appreciated as a desirable source of milk protein. It has the special advantage of being available in many varieties. Also, processors and distributors have made it available in convenient, attractive, consumer-size packages. The buyer's response to these inducements is reflected in increased sales (Table 1).

Routine Flavor Examination of Milk Products

Regrettably, too many milk processors and distributors are apprised of the flavor of their milk by consumer complaint. Furthermore, these same distributors will never know what percentage of their milk customers are on the verge of complaining, but never do, when the flavor is not to their liking. Fortunately for the processor, these customers continue to take what comes, having the fullest confidence in the integrity of their milk dealer.

Good business principles would seem to dictate that the processor should be more familiar with the shortcomings and merits of his products than are his clients. So-called "growth" companies acquaint their customers with the merits of their merchandise so they may get the greatest value and service therefrom. The milk distributor might well do likewise, thus minimizing complaints and at the same time putting into practice some good salesmanship psychology. To this end, a careful systematic plant study of all the milk products sold should be made. Often the perfection of quality in such "minor" items as buttermilk and cottage cheese is responsible for more milk sales. These products enable one to get the foot in the door —and then to put milk into the refrigerator. The scoring routine should include examination of the fresh and aged products as well as those under ideal and adverse conditions. All examinations need not be made daily, but they should be made periodically.

A plant manager contemplating a regular milk product examination program should:

a) Delegate responsibility for flavor control upon competent personnel who know the basic causes of off-flavors in milk;
b) Provide suitable facilities for storing, tempering, and judging dairy products;
c) Require routine examination of all the milk products;
d) Include competitors' products, repackaged so their sources are unknown to the judge;
e) Have a competent company judge go over all the products occasionally; and
f) Make more than lip-service use of organoleptic findings.

Tracing the Causes of Off-Flavors in Milk

The literature is filled with references to the off-flavors, odors, taints, and tastes of milk. One is overwhelmed at the stupendous task of trying to classify and describe these flavors. Nevertheless, a careful study suggests the possibility of grouping them according to the primary cause of the defect. Accordingly, the following classification seems to be all-inclusive: *(a)* bacterial growth, *(b)* feed, *(c)* absorbed, *(d)* chemical composition of milk (mastitis), *(e)* processing and handling, *(f)* chemical (hydrolytic and oxidative) changes, and *(g)* foreign material.

Distinguishing Characteristics of Off-Flavors in Milk

Each of the causative groups of off-flavors in milk has some distinguishing characteristics which aid in the identification or elimination of the source of the defect. These characteristics, presented in Table 2, are well known by the milk judge, who makes use of them regularly in diagnosing milk flavor troubles. An explanation of some of the characteristics of off-flavors falling into certain causative groups is necessary.

TABLE 2

Distinguishing characteristics of off-flavors in milk

Cause of off-flavor	Chief distinguishing characteristics of off-flavors
Bacteria growth	Unusually high bacteria count: 32,000,000 to 305,000,000 per ml. (Hammer and Hix, *4*) 1,300,000 to 160,000,000 per ml. (Gould and Jensen, *3*)
Feed	May be low-bacteria count; flavor present when milk is drawn; usually worse in night's milk; cows have had access to roughage too recently before milking; very pronounced odor.
Absorption: Direct	Encountered very infrequently; results only after long exposure to very odoriferous atmosphere; odor not present when milk is first drawn.
Indirect, through cow breathing foul air	May be low-bacteria count; odor often suggests uncleanliness; odor present when milk is first drawn.
Chemical composition of milk	Flavor defect is noticeable when the milk is first drawn; milk usually distinctly salty, inherent to individual animal, rarely in mixed milk; animal in advanced lactation or has infected udder.
Processing	Pasteurized, "heated" or modified "cooked" flavor. Detectable immediately after processing; usually not very intense; tends to disappear upon storage.
Chemical: Enzymatic and catalytic	Not present when milk is first drawn or pasteurized; develops readily at low temperatures—below 40° F.; low bacteria count; stored 24 to 72 hours. Three types: a) Rancidity—in raw milk and in improperly processed or raw milk–contaminated homogenized milk; bitter, soapy; defect worse in cream than in milk and more intense in butter than in cream. b) Oxidized—chiefly in pasteurized milk; papery, tallowy. c) Activated—in homogenized milk exposed to light; suggests burnt protein.
Foreign material	Defect present in freshly bottled product; rarely increases in intensity upon storage; taints varied as brine, medicinal, paint, fly spray, etc.

a) Bacteria. A study of the early literature on milk flavors is indeed confounding, for every taint generally was considered to result from bacterial growth. Such may not have been the case. Yet, owing to lack of or inadequate refrigeration, the assumption was likely to be correct. Of one thing the taster may be sure: If the off-flavor is due to bacterial growth, the numbers of bacteria per milliliter are well up into the millions. Frequently, a taster, suspicious that a sample of milk is sour, will run Mann's acid test on the sample to verify his suspicion. The results are often misleading and actually furnish little evidence of value in determining the source of the trouble. The average taster can readily detect developed acidity of the order of 0.01%. If the test revealed an acidity of less than 0.20%, which it generally does, the taster may erroneously assume that the milk is sweet; thus, his suspicions are allayed. Actually, the milk may be sour at 0.17% acidity. It is the by-product of the developed acidity, not the normal, acid-reacting constituents of milk, which is tasted. Thus, a normal milk of 0.15% acidity will yield an acid taste upon bacterial growth long before a definite percentage of acidity attributable to souring is reached. Seldom will the taster be in error in alleging a milk flavor defect to be caused by bacterial growth if the numbers of bacteria per milliliter of the sample are beyond a million. However, it is hazardous to ascribe a flavor defect of milk to bacterial growth when the bacterial population is low. Milk judges soon learn that certain off-flavors are always the result of bacterial growth and can diagnose the off-flavor problem with assurance.

b) Feed. Probably the chief distinguishing characteristic of feed flavors in milk is their dissimilarity to the odor or taste of the contributing feed. Frequently, the flavor is of such a nature as to give no hint whatever of the causative agent. Thus, like a herring dragged across the trail, feed flavors often will lead one to false premises. For example, the soda-like, unclean taste sometimes noted in milk suggests rank contamination with washing powder or neutralizer, the real cause possibly being alfalfa pasture. Also, the fishy taste of milk conceivably might well be attributed to the feeding of fish meal, whereas proper feeding of fish meal does not result in fishy milk. Rather, fishiness may result from feeding beets and beet tops. A classic piece of research by

English workers showed that the betaine in beet tops, on reaching a certain level in the milk, became oxidized to form trimethylamine, a chemical substance having a distinctly fishy flavor.

Other characteristics of feed flavors are: The flavor is generally accompanied by an odor; the flavor is present when first drawn; and, generally, the flavor is more pronounced in the night's than in the morning's milk.

c) Absorbed. A characteristic of the absorbed odors in milk is their ability to yield the same olfactory sensation as the atmosphere in which the milk was exposed. Absorbed flavors may get into the milk directly from the surrounding air or indirectly through the body of the cow. Directly absorbed odors require a relatively long absorptive period; indirectly absorbed odors, by interchange of the odoriferous substance in the breathed air with the blood as it is oxygenated in the lungs, take less than a minute, or the time required for blood to make the circuit through the body of the cow. Thus, the stench of the stable and the pungency of old silage in the fouled air of the milking area will be detected in the milk at once, even in machine-drawn milk completely out of contact with the air. Usually absorbed odors do not change their nature in the milk and are easily identified.

d) Chemical composition. With regard to composition, milk has the best flavor when the percentage of lactose is high in respect to the chloride content. At the onset of udder troubles, e.g., mastitis, milk secretion is abnormal. However, nature tends to maintain a constant osmotic pressure in the body fluids. To this end the mol concentration of lactose decreases and that of sodium chloride increases. Consequently, shifts are made to the lactose concentration in favor of the chlorides. As a result, mastitic and late-lactation milk is often distinctly salty.

e) Processing. The flavors of milk resulting from heat are quickly perceived by tasting. Gould's *(2)* classic work shows that the cooked flavor is formed upon heating milk to a specific time-temperature exposure. For example, momentary heating of milk to 76°-78° C. invariably produces a cooked flavor. Slightly lower temperatures for a longer period have similar effects. Cooked flavors are usually easily recognized immediately after heat treatment.

f) Chemical changes of hydrolytic and oxidative nature.

1. *Hydrolytic.* The chief characteristics of flavors resulting from fat hydrolysis are their repulsiveness and their delayed taste-reaction time. The rancid flavors are always associated in one way or another with raw milk, whether nonpasteurized, inadequately pasteurized, or contaminated with raw milk after pasteurization. Since homogenization accelerates lipase activity, careless processing and handling of homogenized milk that permits contamination with even small amounts of raw milk or cream results in rancidity.

2. *Metal-induced oxidation.* The distinguishing characteristics of metal-induced oxidized flavors are their quick taste-reaction time; their resemblance to cardboard or tallow; and their association with high quality, pasteurized, nonhomogenized milk. Rarely will this type of flavor be found in homogenized milk.

3. *Light-induced oxidation.* The distinguishing characteristics of activated flavors are: the high incidence of association with homogenized milk, their relatively quick perception; and their very slight suggestion of burnt milk. Consumer complaints on this flavor are common. Frequently, children will complain that the milk "tastes like medicine." Usually the flavor can be associated with glass containers, with homogenized milk, and with exposure to light. A check on the control plant–stored sample will reveal a freedom from this specific off-flavor.

g) Foreign. So-called foreign flavors in milk are few. Usually they are distinctive in character, suggest a specific substance, and are readily detected. For example, turpentine, kerosene, fly spray, tar, and paint, all foreign to milk and its degradation products, sometimes get into milk. Generally the source of the flavor trouble is easy to trace.

Securing Clues to the Possible Cause of Milk Flavor Defects

The cause of the off-flavor first must be sought if a flavor defect is to be corrected. To this end the dairy products judge attempts to seek the answer to a number of questions, such as:

What is the taste of the milk?
Are customer complaints occasional or general?
Is the milk raw or pasteurized?
Does the defect occur sporadically or has it persisted over an extended period of time?
Is the defect present when the milk is first drawn?
How soon after production is the defect noted?
What is the bacterial count of the milk?
Does the defect occur in mixed milk or only in milk from an individual cow?
To what kind of roughage have the cows had access?
Has the milk come in contact with copper equipment?
Has the milk been held in the refrigerator for more than 48 hours?
Has every-other-day delivery aggravated the problem?
Was the milk homogenized?
Was the milk packaged in glass or in paper containers?
Has the milk been exposed to sunlight?
Does the same flavor show up in the plant-control samples?

In seeking answers to these questions the examiner must be mindful of the facts *(a)* that certain flavors are present only in raw milk and

in homogenized milk improperly protected or contaminated with raw milk, for example, rancidity; *(b)* that before bacteria bring about flavor changes their numbers must be in the millions per milliliter; *(c)* that a sudden general outburst of flavor complaints usually indicates contamination with foreign material or raw milk contamination of homogenized milk; *(d)* that a general increasing number of complaints over an extended period indicates an oxidized flavor; *(e)* that troublesome off-flavors resulting from direct absorption are practically nil; and *(f)* that many off-flavors are definitely of seasonal occurrence.

Have We Properly Evaluated Flavor in the Quality Yardstick?

The answer to this question must be "No!" But the trend in the proper evaluation of flavor as a functional part of dairy products' quality control is reassuring.

For too long the flavor of dairy products has been assumed to be right. Even today, some processors examine their product only when consumer complaints arise. Fortunately, the manufacturer of new products often is more aware of the importance of flavor to repeat sales than are processors of older established dairy products, such as beverage milk and butter.

Certain standards of sensory perfection, established for all food, make possible continued sales for specific products. For example, crackers must have their crispness, caramels their chewiness, and nonfat dry milk solids its dispersibility, if these products are to compete favorably with other food. Consumers are often enticed to a particular brand because of its superiority in some organoleptic quality. Many times, whether or not the consumer purchases a product will be determined largely by its flavor.

The dairy industry is forced to place more emphasis on the flavor of its products in order to meet the competition of other foods as well as to meet competition within the industry itself. Granted a safe, nutritious, reasonably priced product, a consistently good flavor will make for satisfied customers—truly the keystone of a thriving, sound industry.

The dairy industry today cannot afford to ignore or minimize the importance of flavor in dairy products. Someone has said that "flavor is the voice of food." Be that as it may, flavor is the bait by which the unsuspecting is lured to approach the "Fountain of Health" among foods—dairy products. Despite the unique nutritive values of milk and its derivatives, these supreme food products contribute nothing to the zip and longevity of life until they are taken into the human stomach.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASES

281. Udskillelse og bakteriologisk effekt af "Tardocillin" ved mejerikredsvis bekaempelse af streptokokmastitis. (Secretion and bacteriological effect of "Tardocillin" in elimination of streptococcal mastitis). H. C. HOVMAND and A. J. OVERBY. Nordisk Veterinaermedicin, 7, 833. 1955. (English Summary).

A slowly secreted penicillin compound "Tardocillin Leo vet." (*N,N'*-dibenzylethylen-diamin dibenzylpenicillin in a mixture of vaselin and paraffin oil with added aluminummonostearate) was used on cows suffering from streptococcal mastitis.

One infusion of 100,000 I. U. of "Tardocillin" per gland maintained therapeutic levels in the udder for 5 days. Sixteen of 21 herds were found negative after one treatment. In the remaining 5 herds only 4 cows had relapses.

"Tardocillin" could be detected in therapeutic amounts in milk from the 12th milking in contrast to penicillin which could not be detected after eight milkings.

"Tardocillin" behaves much like penicillin in milk. It follows the skimmilk rather than the fat and is also inactivated by penicillinase.

T. Kristoffersen

BUTTER

282. Fremstilling af usyrnet smoer. (Manufacture of sweet cream butter). T. JENSEN, G. ALS, H. MADSEN, and E. O. PETERSON, Gov. Research Inst. on Dairy Ind., Hilleroed, Denmark. Research Bull., 96. 1955. (English Summary).

Prompted by the preference of certain countries and the better keeping quality of sweet cream butter, the Research Institute conducted experiments in cooperation with 14 creameries in order to be able to make recommendations for manufacturing of such butter.

In summertime the best body and texture and the lowest fat loss was obtained by cooling the cream to 5-6° C. or lower and holding at that temperature for approximately 20 hours. During the winter the best body and texture was obtained treating the cream by a modified 8-19-16 method. A salt content of at least 1.5% gave the best results.

Unwashed sweet cream butter contained less curd than unwashed ripened cream butter. Washed butter kept as well as unwashed provided it had been properly worked. The use of wooden churns versus metal churns did not seem to influence the keeping quality.

Sweet cream butter deteriorated only little during 6 months of storage at –20° C. Ripened cream butter of equal quality after 2 weeks of storage became strongly oxidized under the same conditions.

Attempts were made to ripen the sweet cream buttermilk to make a consumer-acceptable product. Fairly good results were obtained by inoculating with 5% starter and incubating at 19° C. for about 20 hours. Pasteurization prior to ripening improved the finished product. Homogenization resulted in excessive wheying off.

T. Kristoffersen

283. Ambulante forsoeg med udluftning af floede. (Experiments with aeration of cream). A. N. FISKER and O. LARSEN, Gov. Research Inst. on Dairy Ind., Hilleroed, Denmark. Research Bull., 97, 74. 1955.

Experiments were conducted to determine if aeration of the cream immediately following pasteurization improved the flavor of butter. Two methods of aeration were tested. Neither method of aeration improved the quality of the butter. The ave. score of 20 control samples after 2 and 4 weeks of storage equaled that of the experimental samples.

T. Kristoffersen

284. Churn. H. BLANCHETTE, S. S. DE DRUMMOND, and V. BOLDUC (assignors to La Compagnie J. A. Gosselin, Limitee). U. S. Patent 2,737,368. 2 claims. Mar. 6, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 1: 127. 1956.

A drum-shaped churn so designed that it contains two chambers—one on the edge of the churn and the other in the center. Means of circulating the cream and buttermilk from the outside chamber to the center is provided through channels located within the drive shaft.

R. Whitaker

CHEESE

285. Adjustment of casein fat ratio of milk for cheesemaking. R. M. DOLBY and W. L. HARKNESS, Dairy Research Inst., Palmerston North, N. Z. N. Z. J. Sci. and Technol., **37**, 1, B: 68. 1955.

As milk is piped from bulk storage to cheese vats, a small portion is allowed to bypass and flow through a separator previously calibrated as to its cream test. Separated milk is allowed to flow back into the milk line and cream used to standardize whole milk based on analysis from bulk vat. Standardization tables are given. Accuracy of method was sufficient to give standard deviation of 0.6. G. E. Stoddard

CONDENSED AND DRIED MILKS; BY-PRODUCTS

286. Bread-making composition. R. W. SELMAN, JR. and M. K. LANDCRAFT (assignors to De-Raef Corp.). U. S. Patent 2,736,654. 8 claims. Feb. 28, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 4: 827. 1956.

Bread, containing a considerable portion of whey solids, is improved through the use of 11-19% calcium carbonate and 0.105-0.175% of calcium peroxide. R. Whitaker

DAIRY BACTERIOLOGY

287. Detection of lactic starter inhibitors. C. E. NEAL and H. E. CALBERT, Univ. of Wis., Madison. Milk Dealer, **45**, 5: 53. 1956.

Although there are no chemical tests available, a comparatively simple biological test can be made in 2½ hours which will readily detect antibiotics in milk. The test employs an actively growing lactic culture and 2,3,5-triphenyltetrazolium chloride (TTC). Directions for the test are given. Tables are given showing the concentrations of antibiotics detectable in raw milk using the TTC test and the influence of sanitizing agents on this test.

Advantages of the test over other methods are that the same equipment used for the methylene blue reduction test can be used, and it is sensitive to all the antibiotics that are commonly used to treat mastitis. The test will detect inhibitory substances other than antibiotics which may cause difficulty in the manufacture of dairy products, and can be performed by a person familiar with simple laboratory technique at a low cost. C. J. Babcock

288. A study of coliform organisms. H. B. SIEGMUND, Hendler Creamery Co., Inc., Baltimore, Md. Sou. Dairy Prod. J., **58**, 4: 170. 1955.

Coliform tests of 690 samples of coalesced foam from ice cream mix pasteurized at 165° F. for 30 minutes, plated in five 5-ml. amounts each of 1:10 dilutions, were 97% negative. After the foam was held at room temperature for 24 hours, 54% of 685 similar platings were coliform negative. In a later series, 99¾% of 714 immediate platings resulted in negative coliform tests. Positive plate counts ranged from one to TNTC colonies. All the foam samples were phosphatase negative. The importance of eliminating foam from the pasteurizing vat is emphasized. F. W. Bennett

289. Method of preserving natural rumen microorganisms and product which results therefrom. M. MUHRER. U. S. Patent 2,738,273. 13 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2,374. 1956.

Rumen organisms are mixed with milk and ascorbic acid and freeze-dried to form a preserved form of natural rumen microorganisms. R. Whitaker

290. Bakterieindholdet i nogle befugtningsstoffer. (Bacterial content of some wetting agents). A. MOELLER-MADSEN. Gov. Research Inst. on Dairy Ind., Hilleroed, Denmark. Research Bull., **97**, 116. 1955.

Liquid wetting agents may be a source of contamination of milk and other dairy products in the plant and on the farm.

Some common liquid wetting agents were checked for bacterial content. Ordinarily the wetting agents were sterile on receipt. However, when the bottles were opened and some of the content used daily, an abundant growth of bacteria took place in certain preparations.

Artificial contamination with 10% of surface water and incubation at 30° C. for 5 days resulted in bacteria numbering several millions per ml. of wetting agents in some products. Others showed some bactericidal property. This property appeared to be related to the concentration of the product as it disappeared upon dilution. T. Kristoffersen

DAIRY CHEMISTRY

291. Lactalbumin composition. R. J. BLOCK (assignor to The Borden Co.). U. S. Patent 2,738,275. 4 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2: 374. 1956.

A water dispersible product is produced by mixing from .2 to 4% of gelatin, alginate, alkaline caseinate, or alkaline salts of soy protein with lactalbumin, and milling the mixture. R. Whitaker

DAIRY ENGINEERING

292. In automation emphasis is on instrumentation. J. E. BARBER, Taylor Instrument Co., Rochester, N. Y. Ice Cream Rev., **39**, 9: 54. 1956.

Automation had its early beginnings in the dairy industry when automatic devices were made available for the control of pressure and flow of water and steam and for temperature control of the pasteurization process.

Future developments in the field of automation in the dairy industry may include the intro-

duction of stream analyzers, automatic CIP systems, graphic panel instrumentation, and automatic recording data reduction systems.

When automation is fully developed it is conceivable that the operating crew of the modern dairy plant will consist of one man supplemented by a maintenance crew.

W. J. Caulfield

293. Method of dehydration by freezing. E. P. WENZELBERGER (assignor to The Commonwealth Engineering Co.). U. S. Patent 2,735,779. 10 claims. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**: 3, 604. 1956.

Heat sensitive liquids, such as milk, may be economically dehydrated by this process without injury to flavor, vitamins, and other characteristics. The product to be concentrated is cooled to form a slush of small ice crystals, by maintaining a small (5° F.) temperature differential between the cooling medium and the product and with suitable agitation. The ice crystals are removed and used to precool the raw product supply. A series of cooling steps as described are employed to reach the desired concentration. Economical concentration is realized, as no high vacuum and low temperature cooling medium are required. R. Whitaker

294. Apparatus for standardizing milk. P. HUBMANN (assignor to Package Machinery Co.). U. S. Patent 2,735,614. 3 claims. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 3: 565. 1956.

Details are given for a centrifugal-type milk separator which permits removal of a predetermined amount of fat-rich milk, thus standardizing a stream of milk to a constant lower fat content. R. Whitaker

295. Apparatus for unseating milk can covers. W. WISE. U. S. Patent 2,735,602. 8 claims. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 3: 562. 1956.

A knocker taps the lids of milk cans loose as they move along a conveyor.

R. Whitaker

296. Confection machine. J. B. ORRELL and A. E. WILSHUSEN (assignors to Drumstick, Inc.). U. S. Patent 2,735,398. 4 claims. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 3: 510. 1956.

Construction details are given for a machine which automatically coats the ice cream in factory-filled ice cream cones with chocolate and then with chopped nuts, to form the confection known as "Drumsticks."

R. Whitaker

297. Process and apparatus for continuously freezing confections. E. THOMPSON, JR. (assignor to Emery Thompson Machine & Supply Co.). U. S. Patent 2,735,276. 19 claims. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 3: 479. 1956.

A continuous ice cream freezer is described, in which volumes of air and mix are alternately and proportionately introduced under pressure into a freezer where refrigeration is applied as the air and mix are agitated.

R. Whitaker

298. Adjustable measuring and delivery device for milk or other liquid. L. M. LEVINE (assignor to Mejur Corp.). U. S. Patent 2,736,463. 8 claims. Feb. 28, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 4: 779. 1956.

A volumetric filler for containers of milk consisting of two cylinders containing pistons which alternately discharge into one filling head.

R. Whitaker

299. Fluid cooled injection heater. J. P. TERRETT (assignor to Foremost Dairies, Inc.). U. S. Patent 2,737,374. 5 claims. Mar. 6, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 1: 129. 1956.

A steam injector type of heater for milk and other liquid food products, of sanitary design and equipped with a jacket around the steam injector to permit cooling of the injector.

R. Whitaker

300. Refrigerated milk storage tank and pasteurizer. F. J. ZAMBONI. U. S. Patent 2,738,170. 3 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2: 350. 1956.

Essentially the same as Jour. Dairy Science Absts. of Literature **35**, A58, item #372, 1952.

R. Whitaker

301. Public health aspects of the Roswell pasteurizer. H. WAINESS, Wainess and Assoc., Chicago, Illinois. Ice Cream Rev., **39**, 8: 58. 1956.

The Roswell pasteurizer has been approved and accepted by the U. S. Public Health Service for the pasteurization of milk and milk products at 200° F. with a calculated holding time of 3 sec. A detailed discussion of the major requirements for high temperature pasteurization with an explanation of how these requirements have been satisfied by the Roswell pasteurizer is presented. W. J. Caulfield

DAIRY PLANT MANAGEMENT AND ECONOMICS

302. Automatic merchandising of dairy products. E. E. BROWN, C. D. EVANS, and B. J. TODD, Clemson Coll., S. C. Sou. Dairy Prod. J., **58**, 5: 28. 1955.

Locations of milk vending machines in a men's dormitory and two office and class room buildings were profitable, but the basement of a hotel where a machine was accessible to 125 employees was unprofitable. In all of the locations except the dormitory the ½-pt. carton was the preferred size. Pints were preferred in the dormitory while the ⅓-qt. size was least popular in all locations. When the three prod-

ucts were offered simultaneously, 73.2-86.5% of the sales were chocolate milk, 10.4-22.5% homogenized milk and 3.1-9.7% buttermilk. The limitation to either one product or one size resulted in less total sales although there was some transfer of demand from discontinued items to the item still on sale. Elimination of the "impulse" factor in buying by changing location resulted in a decline of 32.7% in sales. Small changes in prices were a minor factor in making the sales more or less profitable. About 80% of the machine sales in the dormitory were made after the closing of the student canteen at 10 p.m. F. W. Bennett

303. Automation for the present and future in the dairy industry. D. MERLIN, Asst. Ed., Ice Cream Rev., Milwaukee, Wis. Ice Cream Rev., **39**, 9: 54. 1956.

Automation in varying degrees has been practiced in the dairy industry longer than most people realize. Some examples of automation in the processing and handling of milk and milk products are discussed. W. J. Caulfield

304. Reports management must have to maintain a profitable operation. C. A. HUNTER, Harvey B. Hunter Dairies, Charlotte, N. C. Sou. Dairy Prod. J., **58**, 4: 42. 1955.

From his experience as manager of a relatively small dairy, the author lists monthly operating statements (profit and loss), daily butterfat and skim shrinkage, route profit and loss statements and truck cost summary (including costs on each route) as the essential major reports for management. In exercising control, every manager should get and analyze reports, give orders and check them.

F. W. Bennett

305. World casein production shows increase. T. W. MCCABE. Sou. Dairy Prod. J., **58**, 4: 178. 1955.

World casein production in 1954 exceeded 205 million lb., an increase of 27% over 1953 and only slightly under prewar production. Production increased in New Zealand, Argentina, France, Republic of Germany, Italy, and Australia but decreased in Denmark. Production of casein apparently is not increasing in the United States because of a relatively high price on non-fat dry milk solids.

F. W. Bennett

306. Weigh to better profits. A. SANDERS, Natl. Assoc. of Scale Mfgs., Inc., Washington, D. C. Ice Cream Rev., **39**, 8: 52. 1956.

Numerous examples are cited to show how the use of inaccurate scales may adversely affect the profits of the dairy plant. The importance of accurate scales in the receiving, processing, and packaging operations of the modern dairy plant is discussed. W. J. Caulfield

307. Bulk cooling and bulk pick-up. R. L. PEARSON, Craft Cooler Corp., Chicago, Ill. Sou. Dairy Prod. J., **58**, 4: 43. 1955.

Average receiving room costs for milk in cans in the Mid-West range from 16 to 40¢ per cwt. The cost of receiving bulk milk is about 6 or 7¢ per cwt., provided that it is an all-bulk operation. Time of receiving is not as important in the latter case. The farmer may save 11 to 15¢ per cwt. in eliminating stickage, spillage, fat losses, and can costs. The hauler will save about 30-40% of the cost of can pick-up. The quality of the milk may be greatly improved. The plate count of bulk milk should never exceed 25,000 per ml. Care must be taken by the dairyman to avoid contaminating a whole tank of milk. F. W. Bennett

308. Dual job truck tank. P. P. WEIDENBRUCH, Damrow Brothers Co., Fond du Lac, Wis. Milk Dealer, **45**, 5: 98. 1956.

A vacuum type refrigerated wall truck tank proves to be a farm bulk cooling tank on wheels when this dual piece of equipment is put to use at the dairy farm for cooling the milk received directly from a pipe line milker, and then further used for hauling this same milk to the dairy plant. The savings resulting from this use are itemized. C. J. Babcock

FEEDS AND FEEDING

309. Pasture quality and ruminant digestion. I. Seasonal change in botanical and chemical composition of pasture. A. T. JOHNS, Dept. of Sci. and Ind. Research, Palmerston North, N. Z. N. Z. J. Sci. and Technol., **37**, 4, A: 301. 1955.

Pasture ryegrass 12 in. high was cut into 3 sections representing the lower, medium, and upper kinds of the plant. Dry matter increased with height and contained 13.0, 14.5, and 18.0% from lower to upper portions. These three sections contained a total of 40.4, 39.6, and 20% of total dry matter weight respectively. Total nitrogen increased toward upper part while NPN alcohol extract, NPN water extract; NPN and sugar and water extract decreased. Sugar alcohol extract and total sugar showed no consistent trend.

The most striking change in seasonal variation is the rapid fall of protein level in the late spring and rapid rise in autumn.

Standard errors of sampling procedure and chemical estimation have been determined.

G. E. Stoddard

310. Pasture quality and ruminant digestion. II. Levels of volatile acids and ammonia in the rumen of sheep on a high-production pasture. A. T. JOHNS, Dept. of Sci. and Ind. Research, Palmerston North, N. Z. N. Z. J. Sci. and Technol., **37**, 4, A: 323. 1955.

Concentration of total volatile fatty acids, (VFA) individual fatty acids and ammonia in rumen contents of sheep was determined at monthly intervals throughout the year. No

marked change in total VFA or in acid proportions was observed with changing pasture composition throughout the year.

When changed abruptly from hay to pasture, acetic acid proportion of total VFA was dropped markedly whereas butyric acid proportion increased. High acetate percentages were observed on poor quality hay and hay plus crushed oats.

The high protein levels in pasture herbage are reflected in high ammonia levels in rumen contents, ranging from 35 to 130 mg. per 100 ml., but peak levels did not coincide with peak pasture protein levels. The high ammonia levels recorded in winter and early spring were discussed in relation to mineral metabolism of grazing animals. G. E. Stoddard

311. Effect of treatment of dairy pastures with BHC and DDT on the flavor and composition of milk, cream and butter. F. H. McDowall, M. R. Patchell, F. Hurst, and J. M. Kelsey, Dairy Research Inst., Palmerston North, N. Z. N. Z. J. Sci. and Technol., **37**, 2, A: 146. 1955.

Treatment of dry, closely grazed pastures with 2 pounds lindane or 2 pounds DDT per acre three weeks prior to grazing did not taint milk, cream, or butter. Treatment with BHC caused a slight taint to their products. Lindane and BHC appeared in butter at 4-6 and 16-18 p.p.m., respectively. When treated according to recommendations there is no likelihood of New Zealand factory-made cheese and butter containing appreciable proportions of these insecticides. Because of its lower tainting properties DDT appears to be most suitable for treating dairy pastures. G. E. Stoddard

312. Pasture establishment on pumice soils. P. D. Sears, E. O. C. Hyde, and R. M. Greenwood, Dept. of Sci. and Ind. Research, Palmerston, N. Z. N. Z. J. Sci. and Technol., **37**, 2, A: 110. 1955.

In a series of small plots and larger areas, studies were made on seeding and inoculation on light pumice soils. Vigorous early clover growth was best attained by (1) drilling seed ¾-inch deep on well consolidated seed bed with phosphate fertilizer placed close to seed. A roller drill was effective; (2) inoculation of seed by mixing seed with inoculum grown on finely divided peat or pumice or treatment with inoculum suspended in milk. High rates of inoculation are desirable. Proper seeding and inoculation made it possible to reduce rate of seeding and still establish a better and more uniform emergence of seedlings and better seedling survival. G. E. Stoddard

313. Growth of pasture species. II. Perennial ryegrass. K. J. Mitchell, Grasslands Div., Dept. of Sci. and Ind. Research, Palmerston, N. Z. N. Z. J. Sci. and Technol., **37**, 1, A: 8. 1955.

In a study of individually potted plants, the effects of temperatures of 59° F. and 85° F., of full light or shade and of partial defoliation were observed. Under all conditions but shading at 83° F. the increase in number of tillers was faster in perennial ryegrass than in cocksfoot or paspalum. Growth of plant tissue was related to number of tillers. Defoliation reduced tillering and subsequently a reduction in vigor of growth. This was especially true at the higher temperature. Difficulty of applying data to field conditions is discussed.

G. E. Stoddard

HERD MANAGEMENT

314. Milking apparatus. F. J. J. J. Henrard (assignor to Babson Bros. Co.). U. S. Patent 2,735,399. 1 claim. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 3: 511. 1956.

A device for installing in the lid of a milking machine milk reservoir to permit the operator to observe the flow of milk from the teat cups through a transparent window.

R. Whitaker

315. Split ring tubular hand milker device. R. Gaertner. U. S. Patent 2,737,151. 5 claims. Mar. 6, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 1: 71. 1956.

A hand-operated tubular-shaped milker made of flexible material. R. Whitaker

316. Reciprocating grill type barn cleaner. O. Pearson. U. S. Patent 2,737,925. 4 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2: 288. 1956.

Litter is pushed with a series of brackets to the end of the gutter behind cow stalls, the brackets being operated by a reciprocating action. R. Whitaker

317. Milking apparatus. T. W. Merritt, C. A. Thomas, and W. C. Babb (assignors to Babson Bros. Co.). U. S. Patent 2,737,924. 8 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2: 288. 1956.

Details are given for an intermittent inflation type milker. R. Whitaker

318. Vacuum and pulsator equipment for use with the milking of farm animals. G. R. Duncan (assignor to Zero Manufacturing Co.). U. S. Patent 2,737,923. 6 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2: 288. 1956.

A combination pulsator-type milker and mechanically refrigerated reservoir which continuously cools the milk as it comes from the cow.

R. Whitaker

319. Comfort stall structure for cows. A. H. Magness. U. S. Patent 2,737,922. 3 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2: 287. 1956.

A stall for cows with the vacuum pipeline so arranged that the cow is permitted considerable freedom in moving forward and backward in the stall. R. Whitaker

ICE CREAM

320. Automation in ice cream processing. D. A. SEIBERLING, Dept. of Dairy Technol., Ohio State Univ., Columbus. Ice Cream Rev., **39**, 9: 55. 1956.

The possibilities and problems of automation in the manufacture of mix, and in freezing, packaging, and hardening of ice cream are discussed. Achievements in the automation of a medium sized Ohio fluid milk and ice cream plant are outlined. In the author's opinion the greatest obstacle to automation in the dairy industry, is that attitude, "it will not work." W. J. Caulfield

321. Individually packaged frozen confection. D. A. TAYLOR. U. S. Patent 2,735,778. 7 claims. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 3: 604. 1956.

A paper shield for preventing the drip from melting frozen stick novelties from reaching the hand of the consumer. R. Whitaker

322. Flavor mixing valve. H. W. ATKINS (assignor to Dari-Go-Round, Inc.). U. S. Patent 2,736,534. 3 claims. Feb. 28, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 4: 798. 1956.

A valve for introducing flavoring material from a small supply tank into a stream of ice cream being continuously discharged from a freezer. Designed chiefly for soft-serve products such as ice cream, ice milk, milk shake, etc. R. Whitaker

323. Multiflow filler nozzle for plastic comestibles. R. F. ANDERSON. U. S. Patent 2,736,480. 15 claims. Feb. 28, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 4: 784. 1956.

A nozzle for depositing a number of layers of differently flavored ice cream in packages. R. Whitaker

324. Holder and advertising medium for ice cream cones. D. L. LEHMAN (assignor to Belle M. Lehman and Blanche C. Hughes). U. S. Patent 2,736,475. 1 claim. Feb. 28, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 4: 782. 1956.

A four-pointed star-shaped holder for carrying five ice cream cones. R. Whitaker

325. Ice cream flavor inserter. H. W. ATKINS (assignor to Dari-Go-Round, Inc.). U. S. Patent 2,736,274. 4 claims. Feb. 28, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 4: 733. 1956.

A valve is described which permits the continuous introduction of one or more flavoring materials into a stream of ice cream from a continuous freezer. R. Whitaker

326. Dispensing freezer. H. F. SWENSON. U. S. Patent 2,737,024. 15 claims. Mar. 6, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 1: 39. 1956.

A soft-serve type of ice cream freezer with automatic and manually operated electrically-controlled mix feed and ice cream dispensing valve. R. Whitaker

327. Ice cream concentrate. E. G. STIMPSON and R. WHITAKER (assignors to National Dairy Research Laboratories, Inc.). U. S. Patent 2,738,279. 4 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704** 2: 375. 1956.

An ice cream concentrate containing at least 65% total solids. To prevent the lactose from crystallizing, at least 40% of the lactose is hydrolyzed. R. Whitaker

MILK AND CREAM

328. A test for the keeping quality of pasteurized milk. E. A. DAY and F. J. DOAN, Pa. Agr. Expt. Sta., University Park. Milk and Food Technol., **19**, 63. 1956.

A color test using an aqueous solution (0.2%) of neotetrazolium has been devised to measure the keeping quality of pasteurized milk. Approximately 0.5 ml. of milk is placed in a sterile test tube with 0.5 ml. of dye. The tube is evacuated and sealed; placed in an incubator at 37° C. for 4 hours. A pink color in the sample indicates that the milk will undergo flavor spoilage within a 4-day period. The test apparently measures the presence of psychrophilic organisms in the milk. H. H. Weiser

329. Frigofile bakterier i pasteuriseret maelk. (Psychrophilic bacteria in pasteurized milk). P. HEMPLER, Food Control Lab., Odense, Denmark. Nord. Mejeri—Tidsskrift, **22**: 20. 1956.

A survey of 136 commercial samples of pasteurized milk showed psychrophilic contamination in 67.6% of the samples as determined by incubation of plates at 7° C. for 10 days. Only 22.8% of the samples contained coliforms. The author suggests the detection of psychrophils to be a much better measure of post-pasteurization contamination than the detection of coliforms.

Keeping quality tests conducted on pasteurized milk stored at 7° C. showed that freshly pasteurized bottled milk should contain less than 10 psychrophiles per ml. in order to maintain a methylene blue reduction time in excess of 5 hr. after 2 days of storage. T. Kristoffersen

330. A new type home pasteurizer. E. O. HERREID, Ill. Agr. Expt. Sta., Urbana. J. Milk and Food Technol., **19**, 76. 1956.

A new type of an immersion type two gallon home pasteurizer has been developed. It has all the basic principles for heating the milk found in most commercial units. It is important that the raw milk should be free from off

flavors before pasteurization, since the milk is heated in a tightly closed container.

H. H. Weiser

331. Pouring spout attachment. S. J. CARNES. U. S. Patent 2,735,594. 1 claim. Feb. 21, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 3: 560. 1956.

A reusable pouring spout for attaching to a Pure-pak paper milk bottle of the type having an opening in the side of the converging walls, to facilitate the pouring of the contents of the bottle. R. Whitaker

332. Dairy bottle closure. C. L. WENZEL (assignor to Mid-West Bottle Cap Co.). U. S. Patent 2,738,089. 3 claims. Mar. 13, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 2: 330. 1956.

A hood cap for glass milk bottles is described made of .002 in. metal foil, laminated to a thin foldable sheet material. R. Whitaker

PHYSIOLOGY AND ENDOCRINOLOGY

333. A simple method of assessing the moisture extruded through the skin of cattle. R. M. MABON. Nature, **177**, 4502: 284. 1956.

The moisture is calculated from the ratio of the dew point of the air adjacent to the animal's back and the atmospheric dew point taken 3 ft. away and level with its back. The dew point near the animal is taken by an adaption of an Assman psychrometer, consisting of a polished metal box 4 × 4 × 2½ in. with suitable holes to fit over the psychrometer. Care should be taken to keep the wick wet and clean. The hair is clipped over the preferred area, which is over the last rib and near the vertebral column. R. Whitaker

334. Milk consumption studies in the hormonal induction of lactation using identical twin dairy cattle. Puakura An. Research Sta., Hamilton, N. Z. N. Z. J. Sci. and Technol., **37**, 2, A: 88. 1955.

Seven sets of identical twin heifers were assigned to treatments of artificially induced and natural calving induced lactation. Lactation induced by diethyl stilbestrol and progesterone was timed to coincide with calving date of co-twin. Analyses indicated initial secretions of hormonal induced lactations were similar to colostrum with colostral phase lasting longer than it does after normal parturition. Although the total production valves do not in general exceed one-half those of the co-twins treated normally, and the persistency of lactation seemed less, the secretion once established appears to be milk of normal quality

G. E. Stoddard

335. The incorporation of acetate-1-C^{14} into cholesterol and fatty acids by surviving tissues of normal and scorbutic guinea pigs. H. I. BOLKER, S. FISHMAN, R. D. H. HEARD, V. J. O'DONNELL, J. L. WEBB, and G. C. WILLIS, McGill Univ. and Montreal General Hospital, Montreal. J. Expt. Med., **103**, 2: 199. 1956.

There was no significant difference in the rate of incorporation of C^{14} from acetate-1-C^{14} into cholesterol and fatty acids by the liver, adrenal and aorta of scorbutic guinea pigs compared with those of pair-fed control animals. Arterial tissue was noted to be quite active in lipid synthesis. Serum cholesterol and hexosomine in scurvy was confirmed. G. E. Stoddard

336. A research discovery. C. ORR. Hoard's Dairyman, **101**, 5: 250. 1956.

A V-shaped plastic cannula in the duodenum of a Holstein steer, with the tube exposed outside the body wall is described. The method is being used at Michigan State University to sample feed materials after they have been through the stomach of the ruminant.

A. R. Porter

SANITATION AND CLEANSING

337. Automation in dairy processing and cleaning operations. D. A. SEIBERLING, Ohio State Univ., Columbus, and E. J. O'DONNELL, Klenzade Ohio Co., Elyria. Sou. Dairy Prod. J., **58**, 4: 46. 1955.

A completely automatic C. I. P. system of cleaning a HTST pasteurizer has been installed and very successfully operated in an Ohio plant processing 28,000 lb. of milk per day. Automation has resulted in exact control, economy in the use of materials and saving in labor. Similar advantages are anticipated as future automatic processing operations are initiated.

F. W. Bennett

338. Vaskemidler vor mekanisk vask av melkeflasker. (Washing compounds for mechanical cleansing of milk bottles). O. M. YSTGAARD, K. HAARBERG, K. GUNDERSEN, and W. BUE, Dairy Inst., Coll. of Norway, Vollebekk. Bulletin, 47. 1955. (English Summary).

A number of basic and commercially available washing compounds were tested for their effectiveness in cleansing of bottles under both laboratory and practical conditions.

The effectiveness of the compounds were based on (1) the stability of the water film on the interior surface of the bottles, (2) the removal of milk rests as judged by staining with fuchsin and (3) bactericidal effect.

Several combinations of cleaning compounds and a number of commercial preparations were found satisfactory. Based on both cleansing ability and cost a mixture of 10 parts of caustic soda and 1 part of tetrasodiumpyrophosphate was recommended for use.

Satisfactory physical and bacteriological results were obtained with a solution of the above mixture containing 0.5% NaOH. However, a 1% solution increased the physical effectiveness some and resulted in practically sterile bottles.

T. Kristoffersen

339. Method and apparatus for inspecting hollow articles. A. D. BEARD and E. EBERHARD (assignors to Radio Corporation of America). U. S. Patent 2,735,017. 13 claims. Feb. 14, 1956. Offic. Gaz. U. S. Pat. Office, **703**, 2: 404. 1956.

A device is described for automatically detecting dirt and foreign matter in washed milk bottles. A source of diffused ultraviolet light is swept over the interior of the bottle from an inspection head which is lowered into the bottle. The bottle or the head may be rotated. When the reflected light is interrupted by a dirt particle, it alters the output of a photomultiplier. The change in output may be observed by means of an oscilloscope, or amplified and made to reject the bottle by means of a thyratron trigger circuit and reject relay. The reject level may be varied. R. Whitaker

340. Compressing disk assembly for power washing cream separators. W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,738,923. 4 claims. Mar. 20, 1956. Offic. Gaz. U. S. Pat. Office, **704**, 3: 547. 1956.

Details are given for a specially designed disc which is placed on the top of the usual pack of separating discs in a separator. This top disc is capable of compressing the discs below it. The arrangement permits cleaning of the bowl without disassembly. R. Whitaker

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